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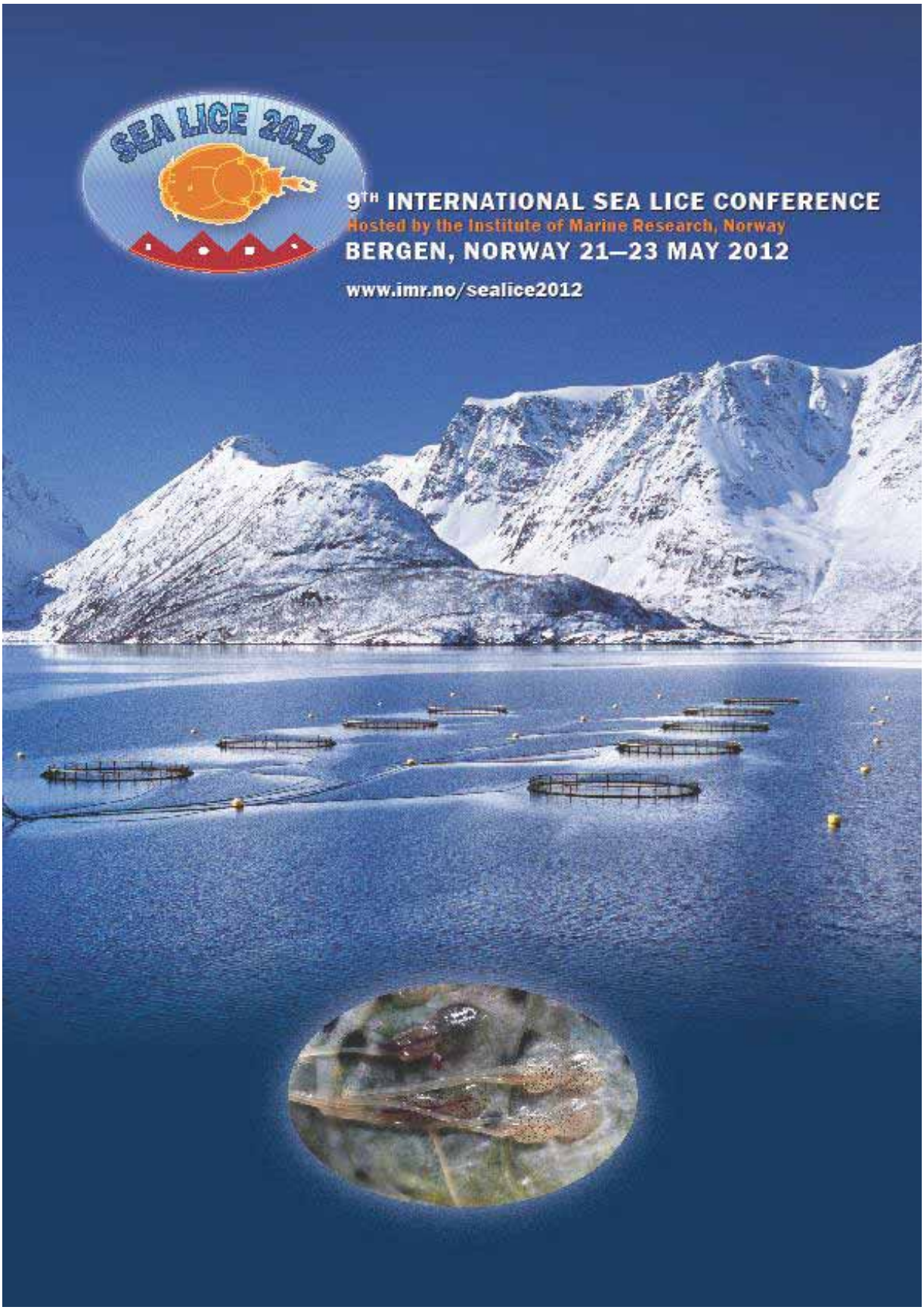
SEA LICE 2012
9TH INTERNATIONAL SEA LICE CONFERENCE
Hosted by the Institute of Marine Research, Norway
BERGEN, NORWAY 21–23 MAY 2012
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11:15 Ian Denholm: Evolution in the fast lane: recent research on pesticide resistance in arthropod pests

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13:30 Fred Page, Les Burridge, Jiselle Bakker, Bill Ernst, Randy Losier, Susan Haigh, Blythe Chang, Monica Lyons, David Wong; Transport and Dispersal of Sea Lice therapeutants used in salmon farms located in southwest New Brunswick, Canada
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16:30 Magne Aldrin and Bård Storvik: Space-time modelling of the spread of sea-lice within and between fish farms

Monday 21 May Evening program

18:00 Reception Fløyen/ Skansen outdoor/ tent (snack and drinks)

Tuesday 22 May Day program

09:00 Fred Whoriskey: Sea-cage farms, sea lice, and wild fish: international lessons from a common challenge

09:45 **Interaction between host - parasite** Chair Simon Wadsworth
Chair intro including poster address

09:55 Simon Jones: Global approaches to understanding diverse mechanisms of resistance to *Lepeophtheirus salmonis* among salmon species

10:15 M.D. Fast, S.E. Friend, S. L. Purcell, J.F. Burka, R.J.F. Markham, A. Donkin, D.B. Groman, J. M. Covello: CPG ODN inclusion in feed induces transient inflammatory responses towards *Lepeophethrius salmonis* and greater protection in Atlantic salmon (*Salmo salar*) following infection and re-infection.

10:30 Linda Jensen, Eivind Larsen, Kai Erik Uleberg, Daniela Pampanin and Fiona Provan: The use of proteomics in sea lice research

10:45 Coffee break in the poster room

11:15 J. Pino, J.L. González Vecino, J. Gonzalez, J. Troncoso, J. Mordue, M. Burkett, A. Quiroz, S.L. Wadsworth: The effect of masking compounds on the disruption and settlement of sea lice (*Lepeophtheirus salmonis* and *Caligus rogercresseyi* on Atlantic salmon (*Salmo salar*)

11:30 Okechukwu O. Igboeli, Sara Purcell, Heather Wotton, Jordan Poley, John F. Burka, Mark D. Fast: Host immunostimulation and its effects on Lepeophtheirus salmonis P-glycoprotein expression and subsequent emamectin benzoate exposure

11:45 J.M. Covello, S.L. Purcell, J. Pino, J.L. González Vecino, J. Gonzalez, J. Troncoso, M. Fast, S.L. Wadsworth: Effects of orally administered immune stimulants on

Atlantic salmon (*Salmo salar*) transcriptional responses and subsequent sea lice (*Lepeophtheirus salmonis*)

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13:00 **Interactions between wild - farmed fish** Chair: Bengt Finstad
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13:10 P. Gargan: Interaction of Sea Lice between Farmed and Wild Salmonids; Potential for Impact, Lessons Learnt and Future Prospects, a Wild Fish Perspective.

13:30 Ove T. Skilbrei, Bengt Finstad, Harald Sægrov, Kurt Urdal, Gunnar Bakke, Frode Kroglund and Rita Strand: Impact of early infestation with the sea louse (*Lepeophtheirus salmonis*) and variability in survival and marine growth of sea ranched Atlantic salmon (*Salmo salar*) smolts 1997 – 2009.

13:45 Pål Arne Bjørn, Ingrid Askeland Johnsen, Rune Nilsen, Rosa Maria Serra Llinnares, Peter Andreas Heuch, Bengt Finstad, Karin Boxaspen & Lars Asplin: The effects of fallowing zones on the distribution and risks of salmon lice infection in wild salmonids in an intensively farmed Norwegian fjord system

14:00 C.C. Pert, and S. Middlemas: Identifying Variations In The Infection Pressure From Parasitic Sea Lice In A Scottish Sea Loch Containing Salmonid Aquaculture.

14:15 Øyvind Knutsen, Ingrid Ellingsen, Lionel Eisenhauer and Hans Bjelland: Modelling dispersion of sea lice (*L. salmonis*) in Romsdalsfjorden –implications for wild and farmed salmon

14:30 I.P. Helland, P. A. Bjørn, O. Diserud, B. Finstad, F. Hansen, P. A. Jansen, R. Nilsen, I. Uglem: Modelling sea lice on wild sea trout; effects of environmental variations and density of farmed fish

14:45 Coffee break

15:15 **Treatment and control** Chair Mark Fast
Chair intro including poster address

15:25 Tor Einar Horsberg: Salmon lice: Treatment and resistance

15:40 P.G. Jones, K.L. Hammell, G. Gettinby, C.W. Revie: Resistance development against emamectin benzoate in different life stages of sea lice (Lepeophtheirus salmonis) on farmed Atlantic salmon (Salmo salar)

15:55 Trond Ove R. Hjelmervik, Per Gunnar Espedal, Heidi Kongshaug, Ketil Malde, Tor Einar Horsberg, Frank Nilsen: Gene expression differences in emamectin benzoate resistant and sensitive salmon lice identified by use of microarray and QPCR

16:10 Sonja Saksida, Diane Morrison, Peter McKenzie, Barry Milligan, Elan Downey, Brad Boyce and Alexandra Eaves: Evaluation of SLICE® efficacy on farmed Atlantic Salmon (Salmo salar) in British Columbia

16:25 J.L. Webb, J. Vandenbor, B. Pirie, S.F. Cross, S.R.M. Jones, S.M.C. Robinson, and C.M. Pearce: Effects of temperature, diet, and bivalve size on the ingestion of *Lepeophtheirus salmonis* larvae by filter-feeding shellfish

16:40 Myron Roth, Randolph H. Richards, Christina Sommerville and Gordon Rae: Toxicity and sub-lethal effects of chemotherapeutants to chalimus stages of the salmon louse, *Lepeophtheirus salmonis*.

16:55 Poster session (light refreshments and bar)

19:00 Conference banquet with host Harald Gjein (CEO, NFSA)

Wednesday 23 May day

09:00 **Advances in sea lice management** Chair: Gordon Richie
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09:10 Randi Grøntvedt: Integrated pest management to control sea lice - are we there yet?
09:30 Kari Olli Helgesen, Tor E. Horsberg: Single dose field bioassay for resistance testing in sea lice (*Lepeophtheirus salmonis*): Development of a rapid diagnostic tool
09:45 M.D. Fast, J. Poley, O.O. Igboeli, A. Donkin, D.B. Groman, H. Wotton, S. L. Purcell: Combinatorial effects of administration of immunostimultory compounds in feed prior to triple dose Slice® (emamectin benzoate) on Atlantic salmon (*Salmo salar*) infection with *Lepeophtheirus salmonis*.
10:00 Erik Høy, Frode Oppedal, Pascal Klebert, Per Rundtop, Ole Folkedal: Mixing of delousing agent in sea cages closed by tarpaulin
10:15 Randi Nygaard Grøntvedt, Bjørn Bjøru, Frode Finne-Fridell, Rune Stigum Olsen, Pundharika Barkved,Vidar Moen and Solveig Gåsø: Delousing in well boats – distribution studies with tracers and pyrethroids

10:30 Coffee break in the poster room

11:00 **Genetics and genomic** chair Frank Nilsen
Chair intro
11:10 Ben Koop: Genomics in Lice and Salmon (GiLS)
11:30 Rasmus Skern-Mauritzen, Ketil Malde, Tomasz Furmanec, Richard Reinhardt, Ben F. Koop, Frank Nilsen: The Salmon Louse Genome Project
11:45 Christiane Eichner, Torbjørn Munkejord Pedersen, Marius Hamre, Ketil Malde, Heidi Kongshaug, Sussie Dalvin, Rasmus Skern-Mauritzen, Lars Are Hamre, Frank Nilsen: Gene regulation in the salmon louse during settlement: similarities in the ecdysteroid regulated gene cascade to other arthropods
12:00 Laura M. Braden, Ben F. Koop, Duane E. Barker, Simon R. M. Jones: Development of a skin injection model for studying local and systemic responses to *Lepeophtheirus salmonis* in salmon.
12:15 Jan Heumann: ABC transporters as factors affecting emamectin-susceptibility of salmon lice (*Lepeophtheirus salmonis*).

12:30 Lunch

13:30 Ben J G Sutherland, Kim W Koczka, Simon R M Jones, Ben F Koop: Stressed lice and refractory responses: Host-parasite transcriptomics

13:45 Rolf B. Edvardsen, Stig Mæhle, Tomasz Furmanek, Ketil Malde, Bjørn Olav Kvamme, Sussie Dalvin, Rasmus Skern-Mauritzen: Transcriptomic analysis of the salmon louse

14:00 E**merging issues C**hair**:** Sonja Saksida
Chair intro

14:10 Frank Nilsen: Novel experimental approaches to facilitate development of new control measure towards sea lice.

14:30 Marit Stormoen, Brad Schofield: Salmon Lice Population Dynamics Modeling with Applications to Treatment Strategies

14:45 P M Hayes and G A Boxshall: Introducing Sealice Explorer – a diagnostic e-taxonomy system for sealice

15:00 Christer R. Wiik-Nielsen, Celia Ridaura and Søren Grove: Search of vaccine candidates against salmon louse

15:15 Lina E.R. Ljungfeldt, Kevin A. Glover, Per Gunnar Espedal and Frank Nilsen: Variability between full-sibling famillies of salmon lice (Lepeophtheirus salmonis) in common garden experiments

15:30 General discussions

Posters

General topics

1) D. Jackson, F. Kane, P. O'Donohoe, T. McDermott, A. Drumm and S. Kelly: Sea lice levels on Atlantic salmon returning to the West Coast of Ireland
2) C. Tröße, H. Kongshaug, L. A. Hamre and F. Nilsen: Characterisation of iron regulatory protein 1 and 2 in the salmon louse (Lepeophtheirus salmonis Krøyer 1837)
3) J. Bengtsen, L. Asplin, P. A. Bjørn and S. Sundby: The salinity tolerance of Lepeophtheirus salmonis larvae
4) O. Samuelsen, A-L Agnalt, B.T. Lunestad, T. Tjensvoll, B. Holmelid, E. Grefsrud, E. Farestveit: The effect of the antiparasitic drug teflubenzuron on non-target species.
5) M. Beattie, A. Bartsch, S.M.C. Robinson, F. Page: Efficacy and Viability of sea lice post treatment with hydrogen peroxide and filtered from well boat discharge
6) S. M. Aaen, J. Bugge, M. French: Hatching of egg-strings exposed to hydrogen peroxide (Interox Paramove 50, Solvay)

Epidemiology

7) M. Nuñez; S. Bravo; M. T. Silva: Behaviour of Caligus rogercresseyi infecting Atlantic salmon (Salmo salar) in a farm located in a new fish farming location in Chile
8) P. Valdes-Donoso, F.O. Mardones, M. Jarpa, M. Ulloa, T.E. Carpenter, A.M. Perez: Co-infection patterns of two high-risk diseases affecting farmed Atlantic salmon in southern Chile (2007- 2009)
9) L. Asplin, A. D. Sandvik, I. A. Johnsen, V. Sundfjord, J. Albretsen, P. A. Bjørn, K. Boxaspen and J. Aure: Fluctuations in the physical climate of the Hardangerfjord and its influence on salmon lice distribution
10) L. Asplin, H. A. Svedberg, P. A. Bjørn, R. Nilsen, R. M. S. Llinares, A. D. Sandvik and M. Myksvoll: Validation of a salmon lice dispersion model in a northern Norwegian fjord

Interaction between host – parasite

11) E. Jakob, T. Sweeten, W. Bennett and S. R. M. Jones: Susceptibility of juvenile sockeye salmon Oncorhynchus nerka to infection with Lepeophtheirus salmonis.
12) C. R. Wiik-Nielsen, C. Ridaura and S. Grove: Phage display – a method for identification of immune inhibitory factors in salmon louse
13) F. Benedikt, C. Dave, B. James, M. Herve: Comparative study of the susceptibility of triploid and diploid Atlantic salmon to infection with the sea louse (Lepeophtheirus salmonis).

Interactions between wild - farmed fish

14) T. Adams: Connectivity and population modelling of sea lice in a Scottish sea loch
15) K.V. Vollset, B.T. Barlaup, U. Pulg, S-E. Gabrielsen: Collection of pelagic stages of salmon lice in a Norwegian Archipelago

16) N.K.G. Salama, B. Rabe, A.G. Murray: Modelling sea lice dispersal in a Scottish fjordic system.
17) M. Foreman, D. Stucchi, M. Guo, P. Chandler, B. Kelly, S. Murphy: Modelling Inter-annual and Intra-seasonal Water Property Variations and their Impact on Sea Lice in the Broughton Archipelago, Canada

Treatment and control

18) L.C.Hastie, C.Wallace, M.A.Birkett, O.Jones, A.J.Mordue, G.Ritchie, J.A.Pickett, J.L.Webster and A.S.Bowman: Effectiveness of a semiochemical for sea lice control: Results from small-scale trials
19) D.F. Jimenez, P.A. Heuch, G. Gettinby, C.W. Revie: A new approach for calculating the uncertainty in the effect of topical field treatments against sea lice in salmonids
20) P. Barría, S.L. Marín: Using the red neutral technique to classify Caligus rogercresseyi (Boxshall & Bravo, 2000) condition of live, moribund and dead after sensitivity bioassays to chemotherapeutants.
21) F. Finne-Fridell, S. Alexandersen, N. Steine, E. Wilkinson, B. Melgård and B. Martinsen: Optimizing sea lice treatment with tarpaulin in large cages.
22) R. Martin and O. Hardcastle: Use of Cypermethrin against Sea Lice Caligus rogercresseyi in Chile

Advances in sea lice management

23) R. Stigum Olsen and J. G. McHenery: Betamax stewardship – The science of best practice
24) S.M.C. Robinson, A. Bartsch, M. Luitkus, K. Pee Ang, D. Cleaves, T.A. Lander, C.M. Pearce and J.L. Webb: Studying the early life-history of sea lice in the Bay of Fundy for the purpose of developing alternative strategies to reduce epidemic-level infestations
25) A. Elmoslemany, S. K. Whyte, K. L. Hammell, and C. W. Revie: Sea lice counts on Atlantic salmon farms: Comparing audit and farm staff counts.
26) A. Ingvarsdottir, F. Provan and H. Bredal: Seafarm Pulse Guard (SPG): Protecting farmed salmon from sealice.
27) F. Oppedal, P. Klebert, E. Høy, R. E. Olsen, L. H. Stien and O. Folkedal: Topical delousing in seacages – salmon behaviour, water movements and therapeutant mixing
28) A. M. Lien, Z. Volent, E. Lien and L. M. Sunde: Shielding skirt for prevention of sea lice infestation on salmon in cages - Experimental study on mooring loads and net deformation.

Genetics and genomic

29) S. Dalvin, B.O. Kvamme, Mæhle, S. and R. Skern-Mauritzen: Transmembrane proteins in the intestine of salmon louse
30) J. S. Leong and B. F. Koop: A Pacific Sea Lice Reference Genome
31) D. Minkley, B. Koop and J. Leong: Composition and high abundance of transposable elements in Atlantic and Pacific Lepeophtheirus salmonis genomes

32) S. Carmichael, A. Tildesley, J. Ireland, J. B. Taggart, P. Skuce, J. E. Bron and A. Sturm: A study of gene expression in Atlantic Lepeophtheirus salmonis populations with differing susceptibilities to emamectin benzoate.
33) S. R. M. Jones, B. J. G. Sutherland and B. F. Koop: Biological and transcriptional consequences of infection with Facilispora margolisi (Microsporidia: Crustaceacida) following vertical transmission among larval salmon lice Lepeophtheirus salmonis.

Emerging issues

34) M. Patricio, S. Rodrigo, M. Carlos, B. Catalina and T. Jaime: A new copepod-specific trypsin mutant as an antigen candidate for a vaccine against sea lice.
35) K. Olli Helgesen, R. Landsem and T. E. Horsberg: Influence of different materials on the concentration of delousing agents in seawater
36) O.O. Igboeli, M.D. Fast, J.F. Burka: Characterizing the interaction of emamectin benzoate with P-glycoprotein in sea lice, Lepeophtheirus salmonis
37) J. G. McHenery: Learning from the past and building for the future: The control of sea lice

Abstracts

Opening

Atlantic salmon – status and threats

Torbjørn Forseth

In this introduction talk on Atlantic salmon I outline the population status of Atlantic salmon across its distribution area, but with particular emphasis on the situation in Norway, a core area for this species. I describe the general trend in abundance and discuss the major causes for the decline. The methods and impacts of the recently implemented management scheme based on population specific spawning targets are presented. The major anthropogenic pressures on Norwegian salmon populations are described and ranked according to their potential for causing further damage. Finally, as a background for the coming conference discussions on impacts of sea lice I summarize the knowledge on population regulation in Atlantic salmon, essential for understanding the potential for population level effects.

Evolution in the fast lane: recent research on pesticide resistance in arthropod pests

Ian Denholm

Problems posed by the evolution of resistance to chemotherapeutants in sea lice parallel ones that have long been encountered in other arthropod pests of agricultural, veterinary and medical importance. Resistance has been documented in over 500 species and extends to all major groups of insecticide. Within many species, continued and intensive exposure to chemicals has led to a gradual broadening of resistance phenotypes caused by cross-resistance, whereby a single mechanism confers protection to several (mostly closely-related) insecticides, and by the appearance of multiple mechanisms in the same individual. The mechanisms themselves can be diverse but most commonly involve either enhanced detoxification by enzymes encoded by multi-gene families (eg. cytochrome P450-dependent monooxygenases), or a mutation to the insecticide's target site – usually a receptor protein – that reduces toxin binding without altering its normal function. Our understanding of resistance mechanisms is benfiting hugely from advances in transcriptomics and genomics enabling genes causing resistance to be identified, cloned, sequenced and their expression analysed far more rapidly than was possible in the past. In many cases, resolution of the underlying changes has led to rapid-throughput diagnostics for use in large-scale monitoring programmes. The tactics available for combating resistance are frequently limited by practical considerations but invariably entail reducing or diversifying selection pressures, taking advantage of compounds with contrasting modes of action and/or non-chemical options for control. Numerous organisations and initiatives exist to promote awareness of resistance and to co-ordinate the development and implementation of anti-resistance strategies. Recent developments with resistance research will be reviewed and their relevance to challenges presented by sea lice will be discussed.

General topics

The Economics of Salmon Lice: Putting a Price on Lice

Jay Abolofia, Frank Asche, Atle Guttormsen

Large and highly dense populations of salmon farmed in open water net pens have been shown to harbor large numbers of motile and adult sea lice, at times producing abnormally high levels of infective planktonic larvae. Host fish often display reduced growth, food conversion efficiency and appetite and the grazing behavior of lice may result in host morbidity and increased susceptibility to secondary infections and predation. As farm profits are highly dependent upon fish growth and feeding, salmon lice pose a significant economic cost to farmers and the industry thereby providing a private incentive for farmers to control infection – the Norwegian Research Council states that sea lice now claim up to 10% of the cost of producing Norwegian salmon. In addition, the farm-to-wild transmission of sea lice has been shown to negatively impact wild stocks. Sea lice production may therefore be viewed as a partially internalized externality whereby farmers have a private incentive to reduce on-farm lice counts, albeit unlikely to the extent necessary to fully dissolve the negative impacts of spillover. Thus, government action (in Norway and elsewhere) has been directed to monitor, treat and manage on-farm lice infection as a means to further reduce the impacts of spillover – in effect, putting a price on lice. This paper offers an overview of the economics of salmon lice providing policymakers with a new perspective of the issue and proposing innovative ways to improve management using economic policy instruments.

Transport and Dispersal of Sea Lice therapeutants used in salmon farms located in southwest New Brunswick, Canada

Fred Page, Les Burridge, Jiselle Bakker, Bill Ernst, Randy Losier, Susan Haigh, Blythe Chang, Monica Lyons, David Wong

In southwest New Brunswick, Canada there are limited options for chemical sea lice treatments. In-feed compounds are largely ineffective in this area and there are no fully registered bath treatments available. From 2009 through 2011 infestations of sea-lice in southwest New Brunswick led to the emergency registration of several pesticide products: Salmosan®, a.i. azamethiphos (ER from 2009-2011), AlphaMax®, a.i. deltamethrin (ER in 2009-2010) and Paramove®, a.i. hydrogen peroxide (ER form 2009-2011). Use of pesticides continues to raise concerns regarding potential effects on non-target organisms, In an effort to help assess the potential zones of exposure associated with the use of these therapeutants a series of dye release experiments were conducted. Mixtures of dye and therapeutants were released from commercial scale tarp, skirt and well boat therapeutant treatments using Salmosan®, a.i. azamethiphos, AlphaMax®, a.i. deltamethrin and Paramove®, a.i. hydrogen peroxide. The empirical results from these studies are being used to test and develop transport and dispersal models for these therapeutants. This work is also being combined with the work described by Burridge et al (this conference) to arrive at estimates of zones of exposure and potential impact to non-target organisms. The work will contribute to the Canadian regulatory environmental assessment process for registration of these therapeutants.

Lepeophtheirus salmonis infestations of migrating Atlantic salmon smolts at eight locations in Ireland with an analysis of sea lice induced marine mortality

David Jackson, Deirdre Cotter, Pauline O'Donohoe, Sarah McEvoy, Frank Kane, Tom McDermott, Jonathan White, Suzanne Kelly & Alan Drumm

Sea lice infestation as a source of marine mortality of outwardly migrating Atlantic salmon smolts has been investigated by treating groups of ranched salmon, prior to release, with a prophylactic sea lice treatment conferring protection from sea lice infestation. A number of studies have been carried out using both established ranched populations and groups of hatchery reared fish imprinted for 5-8 weeks in the sites of experimental releases. In this study, results from twenty five releases, from eight locations along Irelands south and west coasts covering a nine year period are reviewed. Both published and new data are presented including a previously unpublished time series. The results of a meta analysis of the combined data suggest that while sea lice induced mortality on outwardly migrating smolts can be significant it is a minor and irregular component of marine mortality in the stocks studied and does not influence the population conservation status of wild salmon stocks in Ireland.

Effects of therapeutants on non-target organisms in southwest New Brunswick, Canada

Les Burridge, Fred Page, Monica Lyons, David Wong and Bill Ernst

In southwest New Brunswick, Canada there are limited options for chemical sea lice treatments. In-feed compounds are largely ineffective in this area and there are no fully registered bath treatments available. From 2009 through 2011 infestations of sea-lice in southwest New Brunswick led to the emergency registration of several pesticide products: Salmosan®, a.i. azamethiphos (ER from 2009-2011), AlphaMax®, a.i. deltamethrin (ER in 2009-2010) and Paramove®, a.i. hydrogen peroxide (ER form 2009-2011). Use of pesticides continues to raise concerns regarding potential effects on non-target organisms, particularly the American lobster (Homarus americanus), a valuable, commercially-fished species in Atlantic Canada. In a series of bioassays we have determined the acute lethality of these formulations to larval and adult lobsters. AlphaMax® is the most toxic of the formulations with 1-h, 24-h and 10-day LC50s in the ng L-1 range, Salmosan® is lethal to lobsters in the µg L-1 and Paramove® is not lethal to lobsters at environmentally relevant concentrations (g L-1). In addition, results of bioassays conducted with water samples collected during operational treatments will be reported. These data have been assessed in context of use patterns and local hydrographic conditions (Page et al. this conference) and will contribute to the Canadian regulatory environmental assessment process for registration of these therapeutants.

Sea louse *Lepeophtheirus elegans* Gusev, 1951 (Copepoda: Caligidae), a pest on the ranched Korean rockfish *Sebastes schlegelii* in Korea

B. A. Venmathi Maran, Seong Yong Moon, Sung-Yong Oh, and Jung-Goo Myoung

Caligid copepods (Crustacea) called sea lice are known to be pests of cultured fish, since they cause serious diseases and economic losses in fish aquaculture worldwide. In Korea, sea cage aquaculture is flourishing for the last two decades, however, studies on sea lice are rare. At

present, the major species cultured in sea cage farms in Korea is the Korean rockfish. The sea louse *Lepeophtheirus elegans* Gusev, 1951 (Copepoda: Caligidae) was found to severely infect the highly prized Korean rockfish Sebastes schlegelii (Hilgendorf, 1880) (Sebastidae) in Korea. Until now, there have been no reports on sea lice infections from cultured Korean rockfish worldwide and hence, it is a new host record. A survey was conducted from June 2011 to February 2012 at the sea ranched Tongyeong Marine Research Center, Gyeongsangnamdo, Korea. We collected 45 ranched Korean rockfish (five per month; ranging from 10 to 26 cm in total length), which were severely infected by L. elegans on the body surface and fins. Adult ovigerous female, male and preadult sea lice were collected from the body surface and chalimus stages from the fins. Prevalence was recorded as 98.8% and the maximum number of individuals per host was 29. Some ovigerous females were incubated at 20°C to obtain nauplii and copepodids. A systematic study on the life cycle of *L. elegans* is still to be conducted. Severe infection of *L. elegans* can cause serious secondary infections on its host. The identified sea louse can be considered as a serious pest in Korea.

Posters

Sea lice levels on Atlantic salmon returning to the West Coast of Ireland

D. Jackson, F. Kane, P. O'Donohoe, T. McDermott, A. Drumm and S. Kelly

The population structure, prevalence and intensity of populations of Lepeophtheirus salmonis have been studied over a period extending from 2003 to 2011. Infestation data was collected from the interceptory drift net fishery from 2003 until it was closed in 2006. From 2010 data was collected from the inshore draft net fishery. In all 29 samples from the drift net fishery and 28 samples from the draft net fishery have been analysed to date. Prevalence of infestation with L. salmonis regularly approached 100% in samples of hosts recovered from the offshore drift net fishery. Abundance was variable both within and between years with a maximum mean abundance of 25.8 lice per fish recorded in 2004. The population structure of L. salmonis on hosts recovered in the inshore and estuarine draft net fisheries was different to that observed in the more offshore drift net samples. There is clear evidence of recent infestation with L. salmonis in the draft net samples.

Characterisation of iron regulatory protein 1 and 2 in the salmon louse (*Lepeophtheirus salmonis* Krøyer 1837)

C. Tröße, H. Kongshaug, L. A. Hamre and F. Nilsen

Host blood, skin and mucous are the basic nourishment of the parasitic life stages of salmon lice. A blood-filled intestine is especially characteristic for adults. The ingested blood contains high amounts of iron. Iron is essential, but toxic in high dosages, and thus blood-feeding parasites like the salmon louse must possess an efficient system to handle the excess iron. Iron regulatory protein 1 and 2 (IRP1 and IRP2) are known to play a crucial role in this process, by regulating several proteins involved in iron transport and storage at the posttranscriptional level, depending on the cellular iron concentration. In order to gain knowledge about iron metabolism/iron handling two salmon louse IRP homologs (LsIRP1

and LsIRP2) have been identified by sequence similarity to known IRPs in other species. In situ hybridisation with sections of adult females revealed that LsIRP1 and LsIRP2 mRNAs are expressed in the ovaries and the oviducts. Transcription levels of LsIRP1 and LsIRP2 in different developmental stages (from nauplius to adult) and in the presence or absence of blood as a feed source (adult stages) will be analysed by real-time qPCR on whole-body total RNA samples. In order to assess the function of LsIRP1 and LsIRP2, double stranded RNA has been produced and injected into pre-adult females for RNAi silencing of these two genes. The RNAi experiment will provide functional data on these two genes in the salmon louse and their significance in relation to iron handling in adult female lice. The data from these experiments will be presented.

The salinity tolerance of *Lepeophtheirus salmonis* larvae

J. Bengtsen, L. Asplin, P. A. Bjørn and S. Sundby

A critical environmental factor determining the salmon lice larvae behaviour is salinity. A conventional hypothesis within sea lice research is that these larvaes do not tolerate salinity under 20. However, the studies that state this have often been conducted in unnatural experimental settings. Our study has examined the diel vertical migration of Lepeophtheirus salmonis larvae in a salinity gradient from 10 to 35 in an experimental setup including large vertical columns (~4 m high). The results show no clear evidence to support the claim that L. salmonis larvae do not tolerate salinity under 20, and we find that some larvae even prefer to stay at salinities down to 10. This will have important consequenses on how we model sea lice distribution and abundance in fjord areas which is characterized with both strong salinity gradients and absolute values ranging from fresh water to 35.

The effect of the antiparasitic drug teflubenzuron on non-target species

O. Samuelsen, A-L Agnalt, B.T. Lunestad, T. Tjensvoll, B. Holmelid, E. Grefsrud, E. Farestveit

To reduce infestation with salmon lice (Lepeoptheirus salmonis, Krøyer) it is necessary to use anti-parasitic drugs. These drugs are administered either orally via feed or used as bath treatment. Flubenzurons (teflubenzuron and diflubenzuron) are orally administered drug that acts by interfering with the chitin synthesis in salmon lice. Since the bioavailability of flubenzurons in Atlantic salmon are moderate and the metabolism low, most of the drugs will be released from the fish as parent compound via feces. This was confirmed in a preliminary study where analyses of fecal material from Atlantic salmon undergoing medication showed concentrations twice the concentration initially in the medicated feed. Flubenzurons are less soluble in water and associate readily with organic particles and once reached the sediment the compound do not readily disappear. Teflubenzuron and diflubenzuron was frequently in use in the late 1990`s until 2001 in Norway. From 2002, emamectin, cypermethrin and deltamethrin were the drugs of choice but due to instances with reduced sensitivity, flubenzurons were reintroduced in 2008 (total consumption of 3441 and 2919 kg for 2009 and 2010, respectively).Although flubenzurons are rather non-toxic to most marine species it is potentially high toxic to any species that undergo moulting within their life cycle including commercially important species like lobster, crab, and shrimp. The amount of published data

on the effect of flubenzurons on non-target species is limited. Therefore a study was performed to investigation the effect of teflubenzuron on juvenile lobster (Homarus gammarus) when given orally in the feed. Results from this investigation will be presented.

Efficacy and viability of sea lice post treatment with hydrogen peroxide and filtered from well boat discharge

M. Beattie, A. Bartsch, S.M.C. Robinson, F. Page

To date and under current industry practices detached lice are not recaptured/filtered from the well boat discharge. For this study a 3m plankton net was secured to the hull of the well boat in an effort to filter sea lice from an overall discharge rate of 30,000 l per minute for a full 12 minutes. The plankton net was removed and immediately placed in a 500 l container with fresh sea water for transport to a laboratory facility. Pre treatment sea lice counts indicated approximately 40 lice per fish. A total of 6000 fish were treated with hydrogen peroxide at 1460 ppm for a duration of 22 minutes. The total count of sea lice captured by the plankton net was estimated at 220,000. A subsample of all sea lice stages were collected for the following observations; hatching success of attached egg strings, detached egg strings, cut egg strings, adult female survival, and preadult/adult male survival and reattachment success. Over 80% of adult females survived post treatment for up to 7 days without a host. After 19 days post treatment without a host, approximately 20% of adult females remained active. Hatch rates were similar for controls and the treated gravid females. Hatch rates were also similar for attached vs. detached vs. loose egg strings of treated gravid females. Slightly fewer preadult/adult male treated sea lice reattached to naïve salmon in 1m tanks than the complementary control group. For all stages of sea lice there was a reattachement rate post treatment of 30% compared to 43% for controls.

Hatching of egg-strings exposed to hydrogen peroxide (Interox Paramove 50, Solvay)

S. M. Aaen, J. Bugge, M. French

Concerns have been raised regarding the hatching capability of eggstrings from salmon lice detached from the fish during treatment with hydrogen peroxide. In a pilot study, one cage with Atlantic salmon (average weight 1.2 kg) was treated with 1500 ppm hydrogen peroxide in a well-boat. Salmon lice were collected with a net when the treated water volume was flushed out of the tanks. The eggstrings were removed from adult female lice, and transferred to a flask with clean sea water. Control eggstrings were collected from fish from the same cage immediately before treatment. The eggstrings were divided into immature (unpigmented), medium mature /partly pigmented, and mature (pigmented). They were transferred to hatching chambers for salmon lice and incubated for 14 days at 7.8 degrees Celsius. In the control group, all eggstrings hatched and the nauplii developed to copepodides. On average, 57 copepodides were produced per eggstring. In the test group, the unpigmented eggstrings did not hatch. The pigmented eggstrings hatched to some degree, with an average number of 8 naupliis per eggstring. None of these developed into copepodides. The preliminary conclusion is that this formulation of hydrogen peroxide affects hatching of eggstrings and development of salmon lice larvae significantly.

Epidemiology

The plural of anecdote is data: Using 'Big Lice' to inform key research questions

Crawford Revie - Key note speaker

The area of sea lice research is far from unique in the ecological sciences as a domain in which belief and anecdote are expressed as if they were fact, often in the absence of credible evidence. Early research focused on sea lice biology, taxonomy, life-cycle development patterns and very simple descriptive epidemiology. Even by the mid-1990s as a clearer understanding of sea lice dynamics on salmon farms and their control began to emerge, it was still the case that a number of 'accepted truths' rested on somewhat flimsy foundations. Many of the published reports on sea lice infestation patterns were based on just one farm over a limited period of time or a few sites in a single year. These were not an adequately representative sample to properly describe a complex set of host-parasite interactions, which exhibited the expected range of variation and ecological 'noise'.

Over the past decade the situation has changed significantly. A number of studies have been published involving large-scale sea lice data sets, while others will be presented at this Bergen meeting in 2012. In a number of regions data collection has been coordinated by producer and/or legislative organisations, with a resulting increase in the potential to use collected data sets to more completely understand issue around sea lice management on salmon farms. This talk will highlight some key directions, opportunities and challenges as sea lice data sets continue to grow and become more available to various research communities.

Effects of coordinated production of farmed salmon and sea lice interventions within zones, on sea lice control

Peder A. Jansen, Daniel Jimenez, Anja B. Kristoffersen, Randi Grøntvedt and Peter Andreas Heuch

As of 2010, two areas characterized by intensive salmon farming along the Norwegian coast were divided into smaller management zones, aiming to improve sea lice control. Management regulations enforced periods of coordinated salmon production and synchronized fallowing for all active sites within one or more zones. In this communication, we focus on the development of sea lice infections and sea lice interventions for these two areas, as well as for zones within the areas. Detailed data was collected and included weekly counts of sea lice, number and type of delousing treatments and monthly salmon stock data from active sites. We present trends and fluctuations in sea lice infections and delousing treatments for the two areas, as well as for zones within the areas, over the period 2010 – 2011. By initial exploring of the data, fallowing seems to be an effective intervention to control sea lice levels since infections stay low on stocked fish for extended periods of time post fallowing. However, as fish grow and biomass builds up within zones, high sea lice abundances were seen coincident with generally high sea lice population densities in the autumn. We suggest that these high sea lice densities are the result of high rates of sea lice transmission in dense salmon populations of large fish size.

Individual-based models: A new approach to understanding the biological control of sea lice

Maya L Groner, Ruth Cox, George Gettinby and Crawford W. Revie

Individual-based models (IBMs) have been used extensively in fisheries science and health management. Because they can account for variation in numerous traits among individuals, these models often can explain data or address site-specific detail that deterministic models cannot. One application where IBMs have been underutilized is that of multi-trophic interactions, such as biological control programs. IBMs can incorporate variation in behavior, development and fitness of the control agent, pest and its' host and dynamically link these to environmental and biological conditions. We will discuss the development of IBMs of cleaner wrasse (Labridae) as a biological control agent of sea lice on farmed salmon. Cleaner wrasse graze on sea lice that are parasitizing Atlantic salmon. They have been used on salmon farms in Norway and Scotland, often in combination with other sea lice treatments; however implementation of these programs varies among farms. While anecdotal and limited quantitative evidence suggests that these treatments are effective, there have been few efforts to quantify treatment efficacy or develop explanatory and predictive models. IBMs can flexibly incorporate factors that cannot easily be included in deterministic models, including inter- and intra-specific variation in cleaner wrasse feeding rates, stage-selective feeding and behavior. By optimizing cleaner wrasse efficacy, it may be possible to further decrease the frequency of medicinal treatments necessary to manage sea lice densities to recommended levels. Due to increasing concerns that sea lice will develop resistance to current medicinal treatments, development of more efficacious biological controls is critical.

Distribution, magnitude and directionality of sea lice infestations in southern Chile, 2007-2010

Mardones F.O., Valdes-Donoso, P., Yatabe, T., Carpenter, TE., Perez, AM

Effective control of sea lice needs reliable estimates of the geographical distribution of infestation and the size of population requiring intervention. Since 2007, counting numbers of juvenile and adult's sea lice (Caligus sp.) at the farm level have been reported bimonthly as part of the official program established by the fish heath authority in Chile. Data included information from 546 geo-referenced farms including date, species, biomass, water temperature and salinity, and anti-parasite treatments. Exploratory smoothed maps (Kernel density estimation) were performed to describe the intensity of the process as the mean number of epidemics (counts $\geq$6) per farm in a specific zone associated with on-farm productive characteristics. Applied spatial techniques were used to measure the significance of the average directional spread that was computed considering the vectors that connect each outbreak to its temporal nearest neighbors reported. From 2007 to 2010, the mean number of adult and juvenile sea lice decreased significantly from 9.3 and 5.2 to 1.3 and less than 1, respectively. Zones were characterized by sea lice counts consistently high or low along with a high number of treatments administered. In addition, 6 to 8 zones showed certain directionality of the infection process associated often with timing of stocking fish. The study provides results that will generate new hypotheses, and provide useful information for spatial disease control planning in salmon farming areas of southern Chile.

Spatio-temporal analysis of sea lice infection in wild salmon, British Columbia, Canada

E.E. Rees, M. Krkosek, B. Hargreaves, H. Stryhn, S. Jones, C.W. Revie

British Columbia is one of few places in the world where Atlantic salmon are farmed in waters that provide habitat for large populations of wild salmon. Both the wild and farmed populations in this region are susceptible to infection by sea lice. Given the decline in wild populations over the last few decades in other regions, an active area of research is in assessing whether sea lice infestation on farmed salmon negatively impact wild salmon populations. This paper presents a spatio-temporal analysis of an unprecedented wild salmon dataset. Over 140,000 wild salmon were captured by beach seine net in the Broughton Archipelago during the spring and summer of 2003 to 2009 and assessed for characteristics of salmon and sea lice. We used a multivariable regression analysis to determine spatio-temporal factors affecting the proportion of wild salmon infected with sea lice, per sampling instance, given salmon species and size, and sampling date and location. Our results indicate that the proportion of fish infected with sea lice was greater in catches with a higher proportion of chum salmon. Sea lice infection was greater in 2004, and when fish were sampled in late spring. Furthermore, there were strong spatial trends in the data indicating that fish sampled in some sites were more likely to be infected than in other sites. This work provides a baseline for identifying spatio-temporal patterns in this historic data, which will support more comprehensive analyses of associated risk factors.

Space-time modelling of the spread of sea-lice within and between fish farms

Magne Aldrin and Bård Storvik

We present a stochastic model for the spread of sea-lice within and within fish farms. The monthly number if sea-lice at a farm is modelled as a function of several factor. The most important factors are the number of lice the previous month at the same farm and at neighbouring farms and of the sea distance to the neighbouring farms. Other factors include sea temperature and fish size. The model can be used to study the long-term effect of various strategies for reducing the lice density, by simulating from the models under selected scenarios. The model can thus be regarded as a mathematical laboratory, where the effect of potential preventive measures can be tested before they are implemented.

Sea-cage farms, sea lice, and wild fish: international lessons from a common challenge

Fred Whoriskey

Atlantic salmon farming has become global in scale, and is achieving production values unanticipated by the founders of the industry. In areas where the industry occurs within the range of wild salmonids, sea lice have become production and/or social issues. While the original sea lice infestations in the salmon farming industry came from wild fish, the large numbers of salmon (hosts) on farms provided excellent conditions to multiply sea lice numbers. At various times lice epidemics erupted in the industry in a number of the major salmon farming areas, with major impacts on production. Stringent control measures have for

the most part reduced lice levels on farms to the point that farm production is not jeopardized. However, some lice are always present on the farmed fish albeit at low numbers per individual. The many individual farmed fish, combined with the fixed positions of the farms, have concentrated large numbers of lice in habitats important to native or naturalized salmonids. Lice infestations have been noted on wild salmonids in some of these areas, with the lice impacting the health and survival of the wild fish. While the correlation between lice of farm origin and wild fish declines remains under debate, concerned wild fish interests have mobilized to limit or oppose salmon farming.

Along the East Coast of North America, in the areas where salmon farming occurs, wild Atlantic salmon populations are severely depressed and some are listed as endangered by national authorities. This has heightened concerns about negative interactions with aquaculture. In the East Coast salmon farming industry, troubling indications of resistance of lice to Slice™, the major effective treatment mechanism, have surfaced. For this and other reasons, the industry is attempting to geographically shift production patterns, which is bringing salmon farming into new regions of contact with wild fish, heightening concerns about the spread of impacts.

Posters

Behaviour of Caligus rogercresseyi infecting Atlantic salmon (*Salmo salar*) in a farm located in a new fish farming location in Chile

M. Nuñez; S. Bravo; M. T. Silva

Caligus rogercresseyi is the most important parasite affecting Atlantic salmon and rainbow trout farming in seawater in Chile. After the oubreaks of the ISA virus recorded in Region X from 2007, the salmon industry has been expanding south towards Region XI, where 60% of the Atlantic salmon in Chile is now produced. In parallel with the relocation of salmon production, sea lice infestation was also spreading to Region XI and today C. rogercresseyi is also the most serious threat to the salmon industry in this region. This study presents the results obtained through a year of monitoring on a farm located in the "Las Guaitecas Archipelago" in Region XI (44ºS; 74º W) between September 2007 and August 2008. In addition to environmental conditions, abundance and prevalence were highly influenced by the treatments applied to control Caligus. However, abundance also increased with the opening of the new farms which increased the salmon biomass in the area.

Co-infection patterns of two high-risk diseases affecting farmed Atlantic salmon in southern Chile (2007- 2009)

P. Valdes-Donoso, F.O. Mardones, M. Jarpa, M. Ulloa, T.E. Carpenter, A.M. Perez

Infectious salmon anemia virus (ISAV) is a detrimental disease of farmed salmon that caused a large epidemic in Chile (2007-2009). ISAV epidemics may be exacerbated by sea lice (SL), which increases the susceptibility of infested fish and also could act as biological vector for ISAV. In this work, co-infections patterns of ISAV and SL were assessed from a surveillance data collected by the fish health authority. Sanitary status of ISAV and counting records of SL were registered monthly in all Atlantic salmon farms located in 10th region, from July 2007

through December 2009. Four mutually exclusive categories were assigned to each farm every month: ISAV-SL, ISAV-SL free, SL-ISAV free, and ISAV-SL free. The multinomial model of the time-space scan statistics test was implemented to identify clusters of these four categories. Subsequently, a multivariate regression model was fitted to quantify the extent at which clustering of both diseases was influenced by farm management factors. Clusters of ISAV-SL (n=3), ISAV-SL free (n=1), SL-ISAV free (n=3), and ISAV-SL free (n=1) farms were identified. Most clusters (6/8) were associated with high burdens of SL, and the relation observed/expected cases was always high (3/1) in those with both diseases. There were significant associations ($p<0.05$) between productive practices with clusters that showed co-infections. These results provide evidence that the interaction between ISAV-SL showed to be the most likely pattern of infection during the largest ISAV epidemic. In addition, spatial techniques were able to identify areas where better management practices were associated with better sanitary results.

Fluctuations in the physical climate of the Hardangerfjord and its influence on salmon lice distribution

L. Asplin, A. D. Sandvik, I. A. Johnsen, V. Sundfjord, J. Albretsen, P. A. Bjørn, K. Boxaspen and J. Aure

Salmon lice are a threath to especially the wild salmonid fish stocks in the Hardangerjord with its large number of salmon farms. The distribution and abundance of planktonic salmon lice larvae is governed by the water current, salinity and temperature. We will from observations and numerical model results describe how the variability of the physical climate in the Hardangerfjord influence the distribution of salmon lice larvae within the fjord system. In general, small concentrations of infectious salmon lice copepodids reside nearly all over the fjord from nearly any release position, but the spatial variability of magnitude is large. If there exists a lower transmission dose threshold, it will be possible to separate different regions within the fjord regarding influence of salmon lice from the various sources. We also show how persistent current events as internal waves generated at the coast outside the fjord represent a major transport mechanism for the lice. Most of our findings will also apply to other classes of waterborne pathogenes, as e.g. SAV (PD).

Validation of a salmon lice dispersion model in a northern Norwegian fjord

L. Asplin, H. A. Svedberg, P. A. Bjørn, R. Nilsen, R. M. S. Llinares, A. D. Sandvik and M. Myksvoll

The abundance and spatial distribution of salmon lice in the northern Norwegian fjord Folda was studied in field on wild sea trout caught with gill nets and with salmon smolt in sentinel cages. The dispersion of salmon lice released from salmon farms was simulated with a three-dimensional growth and advection model for salmon lice. We find that the abundance of salmon lice in Folda in 2009 was clearly influenced by the farming activity present in the area. Even if a few salmon lice larvae from a source could be dispersed a long distance, and that the variability is great, the resulting pattern from the model simulations indicates that the majority of salmon lice as a treath for the wild fish in an area were originating from relatively nearby farms.

Interaction between host - parasite

Global approaches to understanding diverse mechanisms of resistance to *Lepeophtheirus salmonis* among salmon species

Simon Jones (Keynote speaker)

The salmon louse *Lepeophtheirus salmonis* is an important pest of economically valuable salmonids in seawater throughout the northern hemisphere. Parasite control on cultured salmon relies on chemical treatments applied in the context of management strategies that are often coordinated within regions. The failure of treatments in regions where the parasite has developed resistance to commonly used therapeutants emphasises the need to develop alternate strategies. The development of efficacious vaccines has been hampered by limited knowledge of parasite antigens that elicit protective immunity and by a poor understanding of factors influencing defence responses mounted by the salmonid host. Laboratory exposure trials indicate a range of susceptibilities to *L. salmonis* among salmon species: juvenile coho and pink salmon are relatively resistant whereas Atlantic and chum salmon are susceptible. Early observations indicate that innate resistance is linked to the speed and intensity of local and systemic inflammatory reactions. Although *L. salmonis* somatic antigens are poorly defined, a dopamine-elicited salivary secretion containing prostaglandin E2 and proteoltytic activity dysregulates the expression of proinflammatory markers. More recently, global transcriptomic studies have begun to identify pathways associated with susceptibility (e.g., cell stress, tissue remodelling, diminished immunological responsiveness) and resistance (e.g., cell motility, somatic growth, immuncompetence) but comparative data are urgently needed. Moving forward requires a internationally-coordinated effort in which the development and adoption of research models including salmon and parasites from defined lineages and standardised analytical methods become widely available tools.

CPG ODN inclusion in feed induces transient inflammatory responses towards *Lepeophethrius salmonis* and greater protection in Atlantic salmon (*Salmo sala*r) following infection and re-infection

M.D. Fast, S.E. Friend, S. L. Purcell, J.F. Burka, R.J.F. Markham, A. Donkin, D.B. Groman, J. M. Covello

Successful host responses to Lepeophtheirus salmonis infection, as seen in coho and pink salmon (*Onchorhynchus kisutch* and *O. gorbuscha*, respectively) have previously been characterized by rapid inflammation and epithelial hyperplasia. However, infections in Atlantic salmon (*Salmo salar*) have been characterized by little to no hyperplastic response, and a biphasic immune response resulting in chronic inflammation as the infection persists. We hypothesized that by strongly inducing inflammatory mechanisms and mucosal responses through CpG administration we might enhance localized inflammatory mechanisms and boost Atlantic salmon responses to L. salmonis, leading to greater protection against infection. Transient inductions in splenic interleukin-1β (IL-1β) and matrix metalloprotease-9 (MMP9) expression were followed by mild to moderate inflammation at the infection site in all CpG fed fish and, in some cases, epithelial hyperplasia. This resulted in nearly 50% reductions in lice infection. Following re-exposure, CpG fed fish again showed transient increases in

splenic IL-1β compared to first infection controls, but no different from previously exposed fish. Despite similar splenic inflammatory responses and skin histopathology to previously infected fish, CpG fed fish showed nearly two-fold greater protection than that observed in previously exposed fish (48.5% vs 27.0% reductions at 7 days post re-infection (dpri); 27.2% vs. 13.1% reductions at 17 dpri, respectively). The enhanced protection of CpG ODN administration to previous exposure was consistent across all body surfaces and will be discussed with respect to enhancement of adaptive mechanisms in the host.

The use of proteomics in sea lice research

Linda Jensen, Eivind Larsen, Kai Erik Uleberg, Daniela Pampanin, Fiona Provan

Health diets that contain immunostimulants and other functional ingredients can strengthen the immune response in Atlantic salmon (*Salmo salar*) and thereby reduce the sealice (*Lepeophtheirus salmonis*) infection levels. This can be used as a part of an Integrated Pest Management and will reduce the need for other treatments To examine the effect of functional feed ingredients to protect against sealice, infection trials were conducted. One control diet and three trial diets containing functional ingredients were produced. The diets were fed to salmon (average weight 215g) for 4 weeks before infection with salmon copeopods. When lice had developed to chalimus III/IV, 90 fish per diet were examined for lice loads. Mucus samples from fish fed the different diets were taken before and after lice infection Proteome analyses, where changes in the protein expression in a diagnostic material are measured have been used as a tool to identify markers in mucus that can be related to intake of functional ingredients. Proteomics was used as a tool to examine which modulators in the feed had a noticeable effect on the protein compliment in the mucus layer of the salmon skin. Mucus samples from 50 fish were analysed using nano LC-MS/MS (LTQ orbitrap) and 530 proteins were identified in total. The different diet groups were examined using multivariate statistical methods to find which proteins have an altered expression level due to the diet. In addition mucus from salmon sampled before and after infection was examined to detect alteration in protein expression due to infection. Results will be presented.

The effect of masking compounds on the disruption and settlement of sea lice (*Lepeophtheirus salmonis* and *Caligus rogercresseyi* on Atlantic salmon (*Salmo salar*)

J. Pino, J.L. González Vecino, J. Gonzalez, J. Troncoso, J. Mordue, M. Burkett, A. Quiroz, S.L. Wadsworth

Sea lice rely on a series of host-specific molecules to locate, identify and successfully attach to salmon. Both *Lepeophtheirus salmonis* and *Caligus rogercresseyi* have advanced olfactory and contact chemoreceptors that are required for accurate identification of these host-specific molecules. The presence of these compounds (kariomones) in salmon mucus, elicit a series of behavioral responses. These include identification ‘off-host’ as well as confirmation and attachment processes ‘on-host’. In addition to mucus, the chemical composition of the skin and flesh of the salmon are also important factors in host selection. The use of in vitro Y-tube, arena studies have been used to demonstrate positive rheotaxis of *L. salmonis* and *C. rogercresseyi* to host-specific molecules. A range of plant-based products were assessed as

potential masking compounds to disrupt the recognition and positive rheotaxis of *L. salmonis* and *C. rogercresseyi* to the host-specific molecules of Atlantic salmon. A number of these plant-based products were found to significantly affect the behavioral responses of both species of sea lice. The most effective plant extracts were combined in commercial feeds, fed to salmon and assessed under in vivo sea lice challenge. Significant differences were observed in subsequent settlement, attachment and survival of sea lice. The results are described and the use of these additional control tools discussed in terms of integrated pest management.
Host immunostimulation and its effects on *Lepeophtheirus salmonis* P-glycoprotein expression and subsequent emamectin benzoate exposure

Host immunostimulation and its effects on *Lepeophtheirus salmonis* P-glycoprotein expression and subsequent emamectin benzoate exposure

Okechukwu O. Igboeli, Sara Purcell, Heather Wotton, Jordan Poley, John F. Burka, Mark D. Fast

Lepeophtheirus salmonis control relies heavily on chemotherapeutants, but reduced efficacy of many treatments and the need for an integrated pest management plan require new and innovative strategies for staying a step ahead of the salmon louse. Resistance to emamectin benzoate (EMB), a major sea lice parasiticide, has been linked to Pglycoprotein (P-gp) expression in the louse. We hypothesized that immunostimulation of the host would reduce expression of P-gp and other potential drug-resistance pathways in the attaching *L. salmonis* and increase EMB efficacy. Atlantic salmon were fed different immunostimulatory or control feeds prior to infection with L. salmonis copepodids. Two weeks post-cessation of immunostimulant-treated feed and after development of all *L. salmonis* to adult stage parasites, EMB was administered at 150 µg/kg fish biomass for 7 days. Contrary to our hypothesis, parasites attached to salmon with a history of immunostimulation prior to EMB treatment exhibited significantly greater survival than those on control feeds. Prior to- and 4 days post cessation (dpc) of EMB feed, EMB concentration in salmon muscle ranged from 177 to 232 ng/ml. Adult female lice from hosts with a history of immunostimulation exhibited 1.5-2 fold higher P-gp expression during EMB treatment (4 days on feed) and there was ~2-fold decrease in P-gp mRNA expression by 4 dpc in male and female L. salmonis from the immunostimulatory groups. We will discuss these results with regards to the interaction of prior host immunostimulation and subsequent timing and success of follow up treatment.

Effects of orally administered immune stimulants on Atlantic salmon (*Salmo salar*) transcriptional responses and subsequent sea lice (*Lepeophtheirus salmonis*)

J.M. Covello, S.L. Purcell, J. Pino, J.L. González Vecino, J. Gonzalez, J. Troncoso, M. Fast, S.L. Wadsworth

Novel methods of controlling sea lice populations are required to increase the sustainability of commercial salmon culture. Additional tools are required to supplement the existing controls, such as medicines and management practices. Modulating the immune response of salmon has been shown to have an important effect upon subsequent sea lice infections. In this study immune stimulants were orally administered to Atlantic salmon prior to a controlled

Lepeophtheirus salmonis challenge. In order to characterize the mechanisms behind *S. salar* differential susceptibility, host immune-gene expression, both prior to and following L. salmonis infection were analyzed. These effects were assessed with microarray and validated with specific qPCR for a number of selected immune genes. Differences were observed between the expression profiles of pro-inflammatory cytokines in the treated compared to non-treated groups of fish, as well as in local (skin) and systemic (anterior kidney) gene expression. Differences in a number of specific gene responses correlated closely with subsequent sea lice infection. Salmon fed the immune stimulant formulations had 50% ($p<0.05$) lower sea lice levels compared to untreated groups. Effects against female lice were enhanced with a 60% ($p<0.05$) reduction observed in these stages. The possible reasons for the differences in lice susceptibility are discussed.

Posters

Susceptibility of juvenile sockeye salmon *Oncorhynchus nerka* to infection with *Lepeophtheirus salmonis*

E. Jakob, T. Sweeten, W. Bennett and S. R. M. Jones

The physiological (haematocrit, plasma osmolality and cortisol) and histological (site of chalimus attachment) responses of juvenile sockeye salmon during infection with *L. salmonis* were monitored in three controlled laboratory trials. Sockeye were exposed to an infectious dose of 100 copepodids fish-1 (Trials 1 and 2) or 300 copepodids fish-1 (Trial 3) resulting in a prevalence of 100% and infection densities of 0.28, 0.16 and 0.35 lice g-1, respectively. No mortalities occurred in Trials 1 or 2, however a cumulative mortality of 73% was observed in Trial 3. Histological examination of the gills revealed severe focal hyperplasia of basal epithelial cells and fusion of secondary lamellae at louse attachment sites. Fins showed partial to complete skin erosion around site of chalimus attachment, without evidence of hyperplasia or inflammatory response. Scale loss and skin abrasions coincided with the appearance of pre-adult stages around 20 days post infection (dpi). Plasma osmolality of exposed fish was significantly higher in Trials 2 (dpi 15 and 36) and 3 (dpi 20) compared to controls. Haematocrit values in exposed fish were significantly lower in Trial 1 (dpi 21 and 28) and Trial 3 (dpi 20). Plasma cortisol was significantly higher in exposed fish at dpi 20 compared with controls (Trial 3). Together these results suggest a high susceptibility of juvenile sockeye salmon to infection with Lepeophtheirus salmonis. However physiological changes and mortality depended on the density of infection and coincided with the appearance of pre-adult stages.

Phage display – a method for identification of immune inhibitory factors in salmon louse

C. R. Wiik-Nielsen, C. Ridaura and S. Grove

Ectoparasitic infections cause mortality and serious economic losses in fish farms. Currently, control of the parasites principally relies on chemical treatments. Alternative solutions, such as vaccination, are urgently needed. This strategy however, requires that suitable parasite

antigens can be identified and used in the vaccines. In the present study, we have undertaken such search for antigens in the salmon louse, Lepeophtheirus salmonis, infecting Atlantic salmon. Our strategy has been to look for 'weak points' in the digestive system of the parasite. The parasite is a blood/mucus feeder, and need mechanisms to protect themselves against harmful substances in the ingested blood, such as complement factors. Using advanced techniques, mainly 'phage display', we detected possible complement inhibitors that might be of value for vaccine development. The technique involves the creation of a parasite protein library expressed on T7Select 10-3b bacteriophages. Through a selection or biopanning process, putative parasite complement inhibitors are selected by exposing the recombinant phages to several fish complement factors isolated from salmon serum that has been isolated using chromatographic methods. A parasite protein that binds to complement factor C3, has been identified.

Comparative study of the susceptibility of triploid and diploid Atlantic salmon to infection with the sea louse (*Lepeophtheirus salmonis*).

F. Benedikt, C. Dave, B. James, M. Herve

The susceptibility of diploid and triploid salmon to sea lice, Lepeophtheirus salmonis, was examined in three independent trials. The three trials comprised a tank trial in Scotland, a tank trial in Norway and a cage trial in Scotland. Results indicated a strong correlation between the size of fish and the number of sea lice attached. Triploid fish, generally larger at smolt stage (Norway: diploid mean weight 313.3 ± 35.9g, triploid 345.6 ± 41.6g; Scotland: diploid 108.7 ± 20.6g, triploid 144.2 ± 22.9g), had higher numbers of infecting lice in tank trials compared to diploids (Norway: $H=2.79$, $p=0.093$; Scotland: $H=13.47$, $p<0.001$). After correcting for the different body sizes, however, no difference in louse density between diploid and triploid salmon smolts was seen (Norway: $H=0.75$, $p=0.385$; Scotland: $H=1.59$, $p=0.207$). The trial in Scotland was continued with a re-infection trial. The re-infection trial showed that diploid salmon had a significantly higher number of fresh chalimus attached following a second infection than triploid fish ($H=13.48$, $p<0.001$), being smaller in body size (diploid 105.5 ± 22.4g, triploid 142.8 ± 25.7g). No clear correlation between first infection severity and re-infection severity was found for individual salmon of either group. Sea lice developing to pre-adults showed no host switching behaviour and / or extreme mortality patterns (Diploids: Spearman's $p=0.996$; Triploids: Spearman's $p=0.996$).

Interactions between wild - farmed fish

Interaction of Sea Lice between Farmed and Wild Salmonids; Potential for Impact, Lessons Learnt and Future Prospects, a Wild Fish Perspective

P. Gargan (Keynote speaker)

There has been much debate over the past two decades regarding the interaction and impact of sea lice (L. salmonis) emanating from marine salmon farms on stocks of wild salmon, sea trout and arctic char along the western coasts of Ireland, Scotland and Norway. The potential for sea lice interaction with wild salmonids is examined from a wild fish perspective and a brief history of this sea lice interaction is set out. Documented examples of sea lice impact on salmonid stocks in all three countries is given and lessons learnt regarding farm management practice and potential sea lice impacts on wild fish are set out. Some national and international issues relating to sea lice management and control are examined and the future prospects for interaction of sea lice between farmed & wild fish are discussed.

Impact of early infestation with the sea louse (*Lepeophtheirus salmonis*) and variability in survival and marine growth of sea ranched Atlantic salmon (*Salmo salar*) smolts 1997 – 2009

Ove T. Skilbrei, Bengt Finstad, Harald Sægrov, Kurt Urdal, Gunnar Bakke, Frode Kroglund and Rita Strand

The impact of salmon lice on the survival of migrating Atlantic salmon smolts was studied by comparing the adult returns of sea ranched smolts treated against sea lice with untreated control groups in Dale River in western Norway. A total of 143 500 smolts were released in freshwater from 1997 to 2009 and in the fjord system from 2007 to 2009, from 1 – 6 releases per year. The age and size distribution of wild salmon from three rivers were also monitored for comparison. The adult recaptures of smolts released in freshwater were 0.8 – 1.7 % from 1997 to 2002, but then declined gradually to 0 – 0.2 % in 2007/2008. This development corresponded with poor marine growth and increased age at maturity of ranched and wild salmon, and indicated unfavorable conditions in the Norwegian Sea. Smolt survival was improved by releasing the smolts at various sites in the fjord system from on 2007 (0.6 – 2.3 % grilse and 2SW salmon). The return percentage of treated smolts was significantly higher than the controls in three of the 35 releases performed; the only release in 1997, one of three in 2002 and the only group released in seawater in 2007. The overall effect of treating the smolts against salmon lice was also significant ($p < 0.05$), but small compared with the variability in return rates between release dates, release sites and release years.

The effects of fallowing zones on the distribution and risks of salmon lice infection in wild salmonids in an intensively farmed Norwegian fjord system

Pål Arne Bjørn, Ingrid Askeland Johnsen, Rune Nilsen, Rosa Maria Serra Llinnares, Peter Andreas Heuch, Bengt Finstad, Karin Boxaspen & Lars Asplin

A critical factor determining the risk of salmon lice infection in an area is the density of farmed salmon. The Hardangerfjord system on the western coast of Norway is intensively

farmed, and has experienced severely increased salmon lice infection pressure on wild salmonids for almost two decades. The Norwegian Food Safety Authority has therefore implemented a special Hardangerfjord regulation. This regulation instructs the fish farmers to perform synchronized fallowing of geographically restricted production zones after a beforehand decided plan. They have also commissioned an evaluation of this regulation, especially with regard to the distribution and risks of salmon lice infection in wild salmonids. The regulation have been preliminarily evaluated by a before-after-control-impact approach combining infection levels in wild sea trout and sentinel caged salmon smolts with a farmed salmon-salmon lice database and a three-dimensional salmon lice growth and advection model. Preliminary results show that the spatial-temporal infection risk on wild sea trout and in sentinel cages clearly has shifted from high risk in outer fjord areas before fallowing (2010) to low risk after fallowing (2011). Concurrently, local biomass densities of farmed salmonids as well as farm levels of salmon lice, have increased drastically in middle fjord areas from 2010 to 2011; and so has the salmon lice infection level in wild sea trout and in sentinel cages.

Identifying variations in the infection pressure from parasitic sea lice in a Scottish sea loch containing salmonid aquaculture

C.C. Pert, and S. Middlemas

Elevated sea lice levels within Scottish sea lochs containing aquaculture have been observed and raised concerns that these sea lice may infect and result in mortalities on salmonid smolt populations in these areas. In this study, a hydrodynamic model coupled with a biological particle tracking model were utilised to determine the possible distribution of infective lice within Loch Linnhe. To measure the infection pressure, 10 sentinel cages were positioned at locations identified by the model from the vicinity of the river mouth to the open sea during May and November 2011 for two, one week deployments, per month. After exposure the fish were removed, euthanized, examined and the number of lice, species and developmental stage recorded. Settlement data show that Lepeophtheirus salmonis copepodid and chalimus I form the majority (33.36% and 55.89% respectively) of settled lice although all stages were identified during the sampling process. Prevalence and intensity on sentinel fish at the 10 locations varied from 5.88% - 63.16% and 1.0 – 1.46 in May compared to 69.39% - 98.57% and 2.15 – 13.40 by November 2011. Preliminary data would suggest that sea infection is generally initiated by the copepodid stage although adult stages were also found.. Infectious L. salmonis were present throughout the Loch Linnhe system during both sample periods although exposure to lice could vary at different locations in the system. However, the infection pressure was considerably higher during November 2011 with levels at some sites having the potential to be detrimental to the health of the host fish.

Modelling dispersion of sea lice (*Lepeophtheirus salmonis*) in Romsdalsfjorden –implications for wild and farmed salmon

Øyvind Knutsen, Ingrid Ellingsen, Lionel Eisenhauer and Hans Bjelland

The goal of the SALMODIS-project is to improve decision support for regulation- and intervention strategies for disease control in salmon farming. The project will develop knowledge and methods to evaluate the cost-benefit of different strategies for disease control

with regards to parameters like economy and wild fish. Romsdalsfjorden will be used as a case study area for both sea lice (*Lepeophtheirus salmonis*) and PD-virus. There exist data on sea lice on site-level from Romsdalsfjorden for several years. We simulate dispersion of sea lice with a high resolution hydrodynamic model (SINMOD) coupled to a population model for lice. A population model has been developed for the pelagic phase of the life-cycle which computes the geographic distribution in the concentration level of the different development stages. The population dynamics is dependent on the egg hatching rate at infected locations, the temperature dependent development rate and the mortality. The egg hatching rate will be estimated from a population model for the parasitic stages of the sea lice for each of the locations. Smolt is simulated as particles released in river mouths that drift outwards with a constant velocity (swimming) in addition to the current. From the coupled model system we then get concentration of sea lice that the smolt travels through on its way out of the fjord and we will use this to estimate infections risk on wild populations.

Modelling sea lice on wild sea trout; effects of environmental variations and density of
farmed fish

I.P. Helland, P. A. Bjørn, O. Diserud, B. Finstad, F. Hansen, P. A. Jansen, R. Nilsen, I. Uglem

Sea lice monitoring on wild salmonids has been conducted in Norwegian fjords since 1992 and yearly variations in sea lice counts have been reported for different regions. However, large scale statistical analyses incorporating multiple years and locations simultaneously have previously not been available. Inclusion of spatial- temporal patterns is important to understand how environmental variables influence density of sea lice. Further, such analyses can be used to check the relationship between intensity of salmon farms and sea lice levels on wild fish. Here, we present results from statistical analyses of sea lice counts of wild sea trout (~4800 individuals) collected in 15 Norwegian fjords in the period 2004-2010. We compare several models based on different measures of sea lice level (e.g. total number of lice, prevalence, lice per weight), environmental variables (e.g. temperature and freshwater runoff) and infection risk from aquaculture (e.g. intensity of farming and lice counts reported from farms). The different models give complementary information, but they also illustrate that the design of the monitoring program is critical to ensure justifiable comparisons. We therefore discuss the ability of such models to explain lice levels on wild sea trout. The outcome of the analyses may be highly valuable for managers and aquaculture industry and is needed to verify effectiveness of mitigation actions and monitoring programs.

Posters

Connectivity and population modelling of sea lice in a Scottish sea loch

T. Adams

Hydrodynamic connectivity between aquaculture sites provides an external source of possible infection by sea lice larvae. This has important implications for the population dynamics of sea lice at the farm sites, and in turn the extent to which these can be controlled by farm managers. We studied a sea loch on the west coast of Scotland, with a view to understanding the population dynamics of sea lice across several farms within it. A hydrodynamic model allows prediction of larval spread around the loch, and estimation of the potential connectivity of the farms. This can either be analysed directly, or used as input to a dynamic population model consisting of several subpopulations. It is found that the spread of lice through the system is strongly dependent on the location of the initial source. Analysis of this model in the light of individual site data is in progress. It is hoped that this multi-stage modelling procedure will allow the temporal and spatial dynamics of sea lice metapopulations to be understood more easily, and assist in the development of approaches to their management.

Collection of pelagic stages of salmon lice in a Norwegian Archipelago

K.V. Vollset, B.T. Barlaup, U. Pulg, S-E. Gabrielsen

Salmon lice, *Lepophtheirus salmonis*, (Krøyer, 1838), is one of the most pressing economic issues in Norwegian salmon industry to date. In addition to the great economical costs to the industry due to mortality, use of medication and increased susceptibility to other pathogens, concerns has also been raised about the impact on wild salmonid populations. High levels of the parasite has been documented in Norwegian fjord systems on both wild migrating salmon smolts (*Salmo salar*) and sea trout that forage in the fjord system. The mechanism of which the salmon lice find new hosts is through a larval pelagic dispersal stages. Documented attempts to quantify and document the distribution of larvae have however been scarce. This is probably a consequence of the difficulties of capturing and identifying them due to relatively low concentration in open waters and the relative number of other plankton of the same approximate size. Here we report results from an ongoing project that attempts to develop reliable methods to capture such stages and identify them. Three methods to assess the concentration of pelagic stages of salmon lice; (1) a surface oriented plankton tow, (2) a network of sentinel cage and (3) a mobile cage system that were towed through the fjord (termed “smoltsimulator”). These were compared to the collection of salmonids in trap nets on a known foraging ground in close proximity to the sampling area. Results from the 2010 and 2011 season are reported in addition to preliminary results from the 2012 season.

Modelling sea lice dispersal in a Scottish fjordic system

N.K.G. Salama, B. Rabe, A.G. Murray

Loch Linnhe is Scotland’s largest fjordic system containing 10 active salmon farms which are consented to produce almost 10% of Scotland’s salmon. The system is separated into two farm management areas (FMAs) where coordinated practices occur. Wild salmonid

populations also exist enabling interactions between wild and farmed salmon. To predict areas of sea lice accumulation and assess the interaction between farms, and between wild salmonids and farms, a coupled biological-physical model is used. This combines a particle tracking component, a biological model describing sea lice development and mortality, with hydrodynamic model output (surface currents). The model is forced with tides, freshwater inputs and realistic winds during validation periods and model predictions are being assessed using data from field sampling of planktonic and settled stage lice during these periods. Preliminary results from the first model validation period during two weeks in May 2011 will be presented here along with a simplified one week run from 2008. Simulations for 2008 and 2011 indicate that larvae can be transported over distances reaching 20-30 km, potentially crossing FMAs before becoming copepodids in elevated but localised concentrations. Copepodids often develop away from their parents' hosts. Using a range of release points representing potential wild salmonid sources indicate varying planktonic lice concentrations occur over a wide spread area. Further model scenarios and validation data will be obtained in 2012 and 2013.

Modelling inter-annual and intra-seasonal water property variations and their impact on sea lice in the Broughton Archipelago, Canada

M. Foreman, D. Stucchi, M. Guo, P. Chandler, B. Kelly, S. Murphy

A high resolution circulation model for the Broughton Archipelago, Canada, is used to simulate the time-varying, three-dimensional velocity, temperature, and salinity fields required by a companion biological model that simulates the transport and development/mortality of sea lice emanating from salmon farms in the region. Hindcasts are carried out for May and May 2008, and May 2010, times of the year when changes in river discharge, solar radiation, and air temperature impact near-surface salinities and temperatures and thus, lice development and survival. Model values are compared with available observations and estimated differences in the resultant lice concentration fields are discussed.

Treatment and control

Salmon lice: Treatment and resistance

Tor Einar Horsberg (Keynote speaker)

Sea lice tolerant to various chemical treatments have become a major problem in most salmon producing countries. Decreased sensitivity has been recorded against most available treatments, like emamectin benzoate, cypermethrin, deltamethrin, azamethiphos and hydrogen peroxide. Although the problem seems to be widespread, the degree of severity varies between regions. To maintain sufficient clearance rates when applying a treatment and at the same time reduce the rate of resistance development, effective non-chemical control strategies are highly needed. When chemical treatments are used, these should be applied strategically and cautiously to obtain maximum effect and minimal selection for resistance. How can this be obtained?

Resistance development is highly correlated with the number of treatments. Resistance can be developed towards a single chemical, a group of chemicals with the same mechanism of action and also simultaneously towards groups of chemicals with different modes of action. This is dependent on the mechanism behind. Thus, different strategies may be needed in different situations. Some general principles for selecting optimal treatment regimens will though apply: 1) Non-chemical control options must be exploited whenever possible. This is especially important when the parasites are fully sensitive! 2) Chemical treatments should be applied strategically. Non-coordinated treatments will have a shorter and only a local effect, shifting the problem between regions. 3) Chemical treatments must have a sufficient effect. Partially effective treatments may speed up selection for resistance. 4) Rotation between different chemicals should be enforced, even when an alternative treatment is more costly or labor intensive.

A critical success factor is a detailed overview of the sensitivity status of the parasites within and between regions. This is currently based on treatment efficacy data, sensitivity data from bioassays and knowledge of water current patterns. New high-throughput laboratory-based sensitivity tests will increase the resolution of sensitivity maps and aid in the selection of optimal control strategies.

Resistance development against emamectin benzoate in different life stages of sea lice (*Lepeophtheirus salmonis*) on farmed Atlantic salmon (*Salmo salar*)

P.G. Jones, K.L. Hammell, G. Gettinby, C.W. Revie

Emamectin benzoate (EMB), an avermectin chemotherapeutant administered to fish as an in-feed treatment, has been used to treat sea lice (*Lepeophtheirus salmonis*) infestations on farmed Atlantic salmon. Recent evidence suggests a reduction in effectiveness in some areas. A major challenge in resistance detection is that occurrence is typically low during the early phase. The objectives of this study were to develop a method for determining differences in the rates of resistance emergence among differing sea lice life stages, to devise a model based on these differences and to explore how such a model might be used to better monitor

resistance development in sea lice populations. This study examined two data sets which contained sea lice abundance data following EMB treatments. One data set was from the west coast of Scotland (2002 to 2006) while the other was from New Brunswick, Canada (2004 to 2008). Data were examined by separating the life stages into two groups: adult females and the remaining mobile stages (pre-adult males, pre-adult females, and adult males). Trends in mean abundance and treatment effectiveness were exploreed for each data set. Findings indicate that resistance emerged at different times between adult females and the other mobile stages. Adult females appeared to develop resistance earlier than other stages. These data suggest that an important part of monitoring for drug resistance in aquatic ecto-parasites may be the need to focus on key life stages.

Gene expression differences in emamectin benzoate resistant and sensitive salmon lice identified by use of microarray and QPCR

Trond Ove R. Hjelmervik, Per Gunnar Espedal, Heidi Kongshaug, Ketil Malde, Tor Einar Horsberg, Frank Nilsen

Salmon lice (*Lepeophtheirus salmonis*) represent significant problems for farmed and wild salmonids. Resistance towards several treatments is emerging, among these Slice®, an in-feed formulation of emamectin benzoate (EB). The mechanism behind this resistance is not yet fully understood. Two separate salmon lice strains were used in the study. One was fully sensitive towards EB, the other displayed reduced sensitivity. Adult female parasites from each population were split into two groups. The first group was exposed to 50 µg L-1 EB for 24 h, the other was left unexposed in clean seawater. A microarray study using a 44k custom-made microarray with around 16,000 genes represented was conducted. A principal component analysis demonstrated that the two strains of lice were grouped separately by their gene expression profiles. Pre-exposure to EB induced a coordinated gene regulation in the resistant strain, but less in the sensitive strain. Among the up-regulated genes with a potential role in resistance development were genes coding for putative detoxification enzymes and an ATP-binding cassette protein. Genes involved in stress responses, energy metabolism and cuticle formation were also differentially regulated. The genes encoding the target proteins of EB (glutamate- and GABA-gated chloride channels) were not differentially expressed, neither was ABCB1, a gene coding for an efflux pump for which avermectins are substrates. No single gene could be identified as causing reduced sensitivity towards EB. The distinctive differential gene regulation between the sensitive and insensitive strain revealed several potential biomarkers for EB-resistance.

Evaluation of SLICE® efficacy on farmed Atlantic Salmon (*Salmo salar*) in British Columbia

Sonja Saksida, Diane Morrison, Peter McKenzie, Barry Milligan, Elan Downey, Brad Boyce and Alexandra Eaves

SLICE® had been a very effective therapeutant for the treatment of sea lice infestations (*Lepeophtheirus* and *Caligus* spp.) in Atlantic salmon farmed in Europe, Chile, eastern North America however in recent years there has been considerable evidence of a decline in efficacy. In vitro bioassays have provided confirmation that sea lice have developed resistance

to the active ingredient emamectin benzoate (EMB). Unlike most other salmon farming regions, SLICE® has been the only therapeutant available for the treatment of sea lice on farmed salmon in British Columbia (BC), Canada, since 2000. In this study, we evaluated whether sea lice resistance to EMB is also developing in BC. Two approaches were taken to examine for SLICE® resistance: 1) examine sea lice and treatment data collected from farms and assess for change in treatment efficacy and 2) determine the effective dose (EC50) in laboratory bioassays by exposing pre-adult and adult sea lice collected from farmed salmon to a range of EMB treatment concentrations in vitro. Since the initiation of this study in the Spring of 2010, data has been collected and evaluated from over 15 farms (approximately 20% of the operating farms). All the Atlantic salmon farming companies and almost all the farming regions were represented in the study. Results suggest that resistance to SLICE® has not developed in BC and that male and female sea lice have different tolerance limits for EMB. Factors as to why the experience in BC appears to differ from other regions will be discussed.

Effects of temperature, diet, and bivalve size on the ingestion of *Lepeophtheirus salmonis* larvae by filter-feeding shellfish

J.L. Webb, J. Vandenbor, B. Pirie, S.F. Cross, S.R.M. Jones, S.M.C. Robinson, C.M. Pearce

Filter-feeding shellfish may act as biological agents in the control of sea lice at salmon farms as a benefit of integrated multi-trophic aquaculture (IMTA). Determining the extent to which various bivalve species ingest sea lice (*Lepeophtheirus salmonis*) larvae under controlled laboratory conditions was an important step toward understanding the potential for shellfish to reduce the number of larvae in the water column at net pens and, from there, investigating whether this affects the number of sea lice on farmed salmon. A series of experiments examined the effects of temperature (5, 10, 15°C), diet (larvae alone, larvae plus phytoplankton), and bivalve size (small, medium, large) on the amount of *L. salmonis* larvae ingested by basket cockles (*Clinocardium nuttallii*), Pacific oysters (*Crassostrea gigas*), mussels (*Mytilus* spp.), and Pacific scallops (*Mizuhopecten yessoensis* x *Patinopecten caurinus*). Bivalves were individually placed in 2-L containers holding approximately 450 sea lice larvae, mostly nauplii, in 750 ml filtered seawater, to assess feeding rates. Results of the temperature/diet experiments indicated that all four species of bivalves ingested sea lice larvae, regardless of phytoplankton being present or not, and that temperature had no significant effect. Data from the size experiments indicated that all three sizes of oysters and scallops ingested sea lice larvae, with large shellfish consuming a significantly greater proportion of the larvae than small individuals. Future research, examining bivalves in the control of sea lice is warranted at a commercial scale at a salmon farm.

Toxicity and sub-lethal effects of chemotherapeutants to chalimus stages of the salmon louse, *Lepeophtheirus salmonis*.

Myron Roth, Randolph H. Richards, Christina Sommerville and Gordon Rae:

We report on the toxicity of several compounds representing different chemical classes to the larval chalimus stage of the salmon louse, *Lepeophtheirus salmonis*. Compounds studied included: azamethiphos (organophosphate), resmethrin (pyrethroid), ivermectin (macrocyclic

lactone) and a propriety compound designated SKB7 (milbemycin). Juvenile Atlantic salmon infected with chalimus were treated via immersion (IM) (1 hour) or intraperitoneal injection (IP) and observed 1 and 5 to 11 days post-treatment. Azamethiphos was marginally toxic to chalimus following immersion treatments (0.1-1.0 mg/l), despite being toxic to fish. Toxicity was not observed in chalimus (or fish) following IP treatments at any of the concentrations studied (0.01-0.1 mg/kg). Resmethrin was not toxic to chalimus following IM treatments (0.05-1.0 mg/l), but resulted in short-term delays in development at higher concentrations. Administration to fish IP resulted in toxicity to both chalimus and fish (1.0 - 4.0 mg/kg), with delayed development observed in chalimus at higher concentrations. Ivermectin was toxic to chalimus (0.001-1.0 mg/l) following exposure via IM, with developmental delays observed at the lower concentrations and toxicity to fish at higher concentrations. Following IP treatment, the compound was found to be toxic to chalimus and fish (0.05-0.5 mg/kg) with delays in chalimus development also being observed. SKB7 was toxic to chalimus following immersion treatment at all concentrations studied (0.01-0.5 mg/l), as well as to fish at 0.5 mg/l. When fish were treated by IP, toxicity to chalimus was observed at 0.5 mg/kg with delayed development observed at 0.05 mg/kg.

Posters

Effectiveness of a semiochemical for sea lice control: Results from small-scale trials

L.C.Hastie, C.Wallace, M.A.Birkett, O.Jones, A.J.Mordue, G.Ritchie, J.A.Pickett, J.L.Webster and A.S.Bowman

Semiochemicals (behaviour-altering odours) are widely and successfully employed in agriculture for the control of insect crop pests. Field trials were conducted at an experimental fish farm in north-west Scotland, in order to determine the effects of a semiochemical, on parasitic sea lice (Lepeoptheirus salmonis) abundance on Atlantic salmon (Salmo salar) hosts. Salmon smolts were maintained in small pens either fitted with a polymer ribbon impregnated with the semiochemical or control pens. The ribbon was designed to release the semiochemical into seawater over an extended period. Three separate trials were undertaken in June-July and November-December 2011, over periods lasting 39, 11 and 11 days, when the fish were exposed to natural sea lice challenges. Prevalence and infection loads of lice observed were consistently lower on fish “protected” than on controls. Prevalence ranged from 24–60% fish infected in controls to 12–35% fish infected in treatments, representing effective reductions of 43–62%. Mean lice load ranged from 0.30–1.19 lice/fish in controls to 0.12–0.52 lice/fish in treatments, representing effective reductions of 43–56%. These results indicate that certain semiochemical substances may offer a degree of protection against sea lice infection in natural and semi-natural marine environments. Further investigations are required, in order to determine the potential value of semiochemical application as a pest management tool in the salmon farming industry.

A new approach for calculating the uncertainty in the effect of topical field treatments against sea lice in salmonids

D.F. Jimenez, P.A. Heuch, G. Gettinby, C.W. Revie

Endemic infections of salmon lice *Lepeophtheirus salmonis* pose a major threat to the sustainability and viability of salmon production. The control of sea lice levels largely relies on the use of delousing agents. Numerous reports in the main salmon producing countries indicate ongoing reductions in efficacy and the development of resistance or at least tolerance of lice to the most effective drugs. However, the current method for evaluation, a point estimate of the effectiveness of the treatment, is inadequate. The nature of field treatments and the variable time-scales involved, mean that reported treatment results have uncertain components. Hence, calculation of a confidence interval is necessary to include a measure of certainty around the true effect of any treatment. In this communication, we will explore a range of sea lice treatment datasets and methods to construct robust estimates and confidence intervals. Treatment effect will be calculated and interpreted at both the pen and site levels. In summarizing the conclusions of this study we will provide recommendations on the most appropriate methods to use. The results of this study will support the design of better guidelines and informed policy decisions for assessing the effect of delousing treatments on salmon farms.

Using the red neutral technique to classify *Caligus rogercresseyi* (Boxshall & Bravo, 2000) condition of live, moribund and dead after sensitivity bioassays to chemotherapeutants

P. Barría, S.L. Marín

Sensitivity of parasite to chemotherapeutants is measured thorough the concentration that affects and kills the 50% of the exposed individuals (EC50 and LC50, respectively). Estimates of EC50 include moribund and dead individuals, but differentiation of moribunds is not simple. The objectives of this study were to compare the percentage of live, moribund and dead parasites *Caligus rogercresseyi*, classified using 3 techniques, and to discuss the usefulness of a more objective technique on parasite classification. Two experiments were carried out. The first experiment compared parasite classification (after being exposed to chemotherapeutants) made by 2 normal vision observers and that by the neutral red technique. Results showed no significant differences between normal vision observers, and that normal vision observers assign a larger percentage of live individuals and a less percentage of moribund than neutral red technique. The second experiment compared classification made by normal vision observer, observer using stereoscopic microscopic and that made by the neutral red. Results showed no significant differences among percentage of individual classified as live by the three techniques. Natural vision observer classified a smaller percentage of moribund and a larger one of dead individuals than the neutral red. Parasite classification should be made using neutral red or stereoscopic microscopic techniques. Since stereoscopic microscopic technique is no different from the normal vision observer it would be more precise if sensitivity parameters are estimated using neutral red. This technique adds complexity to bioassays therefore further validation of parasite classification between the

stereoscopic microscopic and neutral red technique is needed. Acknowledgments: This study was partially financed by Universidad Austral de Chile.

Optimizing sea lice treatment with tarpaulin in large cages

F. Finne-Fridell, S. Alexandersen, N. Steine, E. Wilkinson, B. Melgård and B. Martinsen

Sea lice management has increased focus in the Norwegian aquaculture industry due to wild fish interests and reduced susceptibility to the available sea lice drugs. The maximum number of lice allowed on cultured salmonids is controlled by national legislation. Sea lice are currently treated with different pharmaceuticals in addition to an increasing use of cleaner fish. When using pharmaceuticals it is important to avoid sub optimal treatments as this might induce reduced sensitivity and finally resistance towards the active substance. PHARMAQ has been offered ALPHA MAX® as a deltamethrin based bath treatment against sea lice for more than 14 years in Norway. During recent years the size of the salmon cages has increased significantly. Treatment in large 157 meter circumference polar circle cages requires both skilled personnel and equipment. In order to ensure an optimal distribution of the active substance in treatment tarpaulins, PHARMAQ has investigated the distribution of deltamethrin as a function of time during treatment. In this presentation PHARMAQ will give an update of the ongoing studies with regards to distribution of deltamethrin, comparing different treatment methods and different distribution systems. The aim of the studies was to obtain a best practice for accomplishment of optimal sea lice bath treatments. The results show that distribution of the pharmaceutical is better with the use of tarpaulin compared to skirt, and that the distribution can be optimized by use of improved equipment. Rapid and homogenous distribution of the therapeutic ensures a safe and efficacious treatment of the fish.

Use of Cypermethrin against Sea Lice *Caligus rogercresseyi* in Chile

R. Martin and O. Hardcastle

The use of Cypermethrin (Betamax®) for the control and treatment of Sea Lice Caligus rogercresseyi will be described in a poster presentation. Cypermethrin has been used in Chile since October 2010, as a bath treatment and has been established as an effective method to treat external parasites in farmed salmon and trout populations. Information on the use, number of treatments and number of treated fish will be presented. Efficacy data will also be shown, gathered from farms as part of the product stewardship program, and will differentiate in terms of total numbers and stages of lice, before and after treatment.

Advances in sea lice management

Integrated pest management to control sea lice - are we there yet?

Randi Grøntvedt

The widely used holistic concept of Integrated Pest Management (IPM) has been in use for decades in agriculture. Modern research on the development and integration of several combined pest control measures can be traced back to 1959, when chemical and biological control strategies of an aphid (plant lice) was published. The IPM concept is now well-known and a wealth of IPM strategies and tools for pest-crop cases in agriculture has been published in the scientific literature. The focus seems to be turned from fundamental research on development of several control measures to applied research, where the challenge now is to integrate the complementary control strategies at the research level for creation of additive/synergetic interactions between IPM components.

In contrast to agriculture, use of IPM elements in aquaculture is rudimentary. Sea lice control still rely on pesticides, but the need for long term control of sea lice with a combination of methods is a driver for research and development. Fundamental research proceeds in several areas to develop new tools for sea lice control, including: development of a vaccine, breeding for a more resistant salmon, use of cleaner fish, health feed and several novel technologies. In addition, knowledge on structural measures like year-class stocking and fallowing are increasing. Ongoing research on sea lice epidemiology and monitoring are increasing knowledge on spatial and temporal factors affecting sea lice development. Together with potential new control methods, precision monitoring tools should be further developed to form an expanded IPM "toolbox" to work at local and regional levels. This should be used as a decision support system for selection of sea lice control tactics for a management strategy taking into account interests of producers and impact on the environment.

Single dose field bioassay for resistance testing in sea lice (*Lepeophtheirus salmonis*): Development of a rapid diagnostic tool

Kari Olli Helgesen, Tor E. Horsberg

As part of the project PrevenT, a rapid diagnostic field bioassay has been developed for resistance testing in sea lice. This test consists of protocols for single dose bioassays on three chemotherapeutants used against sea lice: Deltamethrin, emamectin and azamethiphos. Four different strains of sea lice have been chosen for the development, based on their histories of treatment and treatment efficacy. They were all collected from the field and put in continues culture at the NIVA Marine Research Station, Drøbak, Norway. The strains were LS-A (assumed sensitive to all chemotherapeutants), LS-B (assumed resistant to all chemotherapeutants), LS-R (assumed resistant to pyrethroids) and LS-H (assumed resistant to azamethiphos). Two different tests were conducted on each strain to look for resistance: Traditional 6-dose bioassays and small scale treatments. In the search for the single dose of each chemical able to differentiate between resistant and sensitive strains of sea lice, 24-hour bioassays were set up. The concentrations chosen for these dose titration studies were based on results from traditional bioassays and from preliminary testing. The results show a

correlation between traditional bioassays, small scale treatment trials and 24-hour bioassays. This correlation was best for azamethiphos. It is possible to differentiate between sensitive and resistant strains of sea lice based on 24 hour bioassays. The 24-hour bioassays have been used to find tentative breakpoint doses for the classification of strains. The tentative breakpoint doses are used in the on-going field testing of the protocol.

Combinatorial effects of administration of immunostimultory compounds in feed prior to triple dose Slice® (emamectin benzoate) on Atlantic salmon (*Salmo salar*) infection with *Lepeophtheirus salmonis*.

M.D. Fast, J. Poley, O.O. Igboeli, A. Donkin, D.B. Groman, H. Wotton, S. L. Purcell:

We have identified multiple immunostimulatory feeds that have shown the ability to induce protective responses in Atlantic salmon to infection with Lepeophtheirus salmonis. However, even the most encouraging results rarely surpass a 50% protective index in the host. That fact coupled with lessons from single-therapy strategies in the past in human health, terrestrial and aquatic species' culture, make it imperative to identify therapies that can be combined in an integrated pest management approach for sea lice. With this in mind, we hypothesized that immunostimulatory feeds would enhance the protection provided by SLICE® (emamectin benzoate). Atlantic salmon were initially fed one of three different immunostimulatory feeds or a control feed prior to and following L. salmonis copepodid exposures. After removal of the immunostimulatory feeds and 5-6 weeks post infection, Atlantic salmon were placed on a triple-dose (150 µg/kg fish biomass) feed of SLICE® for one week. Although immunostimulatory feeds were successful in reducing L. salmonis levels by approximately 20% over the course of the study, no synergism with SLICE® treatment was observed. These results will be discussed in light of immuno-physiological responses of Atlantic salmon induced by the individual feeds and how they may be affecting L. salmonis populations in this environment.

Mixing of delousing agent in sea cages closed by tarpaulin

Erik Høy, Frode Oppedal, Pascal Klebert, Per Rundtop, Ole Folkedal

Preliminary studies on the distribution of delousing agent in sea cages have revealed a need for knowledge in this topic of sea-lice control. The KMB-project Topilouse contains a work-package concentrating on developing knowledge regarding handling of the tarpaulin and the mixing of water within this type of closed cages. By exploring the driving forces and physics, the aim is to be able to replicate this in hydrodynamic models and thus be able to provide an environment for testing new and existing technology to ensure proper levels of delousing agents and oxygen. During the fall of 2011 there have been done tests at the IMR sea cage facility of Matre in order to reveal some of the dynamics at play in a down-scaled delousing situation. The trials was done in cages of 12x12m with a bright colored, green dye supplied together with the delousing agent in order to directly document the mixing, with a high spatial and temporal resolution. Three treatments was tested in the cages: dye only, dye and the agent in combination, and a control treatment with no fish, only the tarpaulin, distribution system and the oxygen supply system. The treatments was documented by surface video, current measurements (five points within cage and one reference profiler outside), four vertically

profiling CTDs inside cage with fluorescence meters and underwater camera in the center of the cage. A short summary of results show a possible great mixing and current-inducing effect from the fish, and very little effect from the oxygen bubbling.

Delousing in well boats – distribution studies with tracers and pyrethroids

Randi Nygaard Grøntvedt, Bjørn Bjøru, Frode Finne-Fridell, Rune Stigum Olsen, Pundharika Barkved,Vidar Moen and Solveig Gåsø:

An efficient sea lice bath treatment depends on homogenous distribution of the delousing agent in enclosed seawater. In this communication we describe the distribution and achieved consentration of water soluble agents in well boats with enclosed circulating water volumes of 480 and 550 m3, respectively. DNAtracers have previously been used in sea cages for studies of the dispersal of delousing agents. In those studies, DNAtracers demonstrated relatively equal distribution with ALPHA MAX®.As part of the research project Topilouse (A multi-disciplinary effort to improve topical treatments in salmon louse control) DNA tracers were used to study the distribution of pyrethroides in two different types of well boats. They have different dosing systems for delousing agents and different circulation systems. The distribution systems in these two boats were optimized for hydrogen peroxide treatment. In our experiments tracers were mixed with either BETAMAX VET or ALPHA MAX® in a mixing tank before addition to the well. Studies were carried out with and without fish. Our tracer studies show that it takes 7-15 minutes before the distribution in the well is relatively homogenous. The calculated pyrethroidconsentration from DNAtracerswere as expected in the well boats. In contrast, concentration recordings of both ALPHA MAX® and BETAMAX VET indicated that the desired pyrethroidconcentration was not achieved in the wells, neither with nor without fish present. Based on the DNAtracer results, we conclude that the missing pyrethroids cannot be explained by poor distribution in the wells, but is somehow related to the chemical properties of pyrethroids and absorption onto different material surfaces. Results are discussed in relation to an ongoing material study.

Posters

Betamax stewardship – The science of best practice

R. Stigum Olsen and J. G. McHenery

Bath treatments are an essential tool in the veterinary armoury against sea lice and ensuring even dispersion of the medicine has always been pivotal best practice. The increasing size of the cages and the greater quantities of medicine required for treatments might be considered to have increased the risk of transient “hotspot” exposure of the fish. To address this, the industry has developed technologies to ensure the continuing safety of the medicines applied in the baths. As part of the Novartis Animal Health Aqua Betamax Stewardship programme, we have undertaken studies to assess the potential risks of hotspot exposure in terms of fish behaviour and tissue burden of cypermethrin together with more classical ecotoxicological studies. The results from the studies show not only behavioural impacts of exposure but also provide greater insight into the behaviour of the medicine during treatment and likely reductions in the quantities of therapeutant released to the environment.

Studying the early life-history of sea lice in the Bay of Fundy for the purpose of developing alternative strategies to reduce epidemic-level infestations

S.M.C. Robinson, A. Bartsch, M. Luitkus, K. Pee Ang, D. Cleaves, T.A. Lander, C.M. Pearce and J.L. Webb

Sea lice (*Lepeophtheirus salmonis*) at Atlantic salmon farms has been a continuing international problem. Control of aquaculture pests in culture industries has been accomplished with chemo-therapeutants, but there are signs of chemical resistance developing and alternatives are required. Our program is investigating the early life-history of sea lice in the Bay of Fundy to identify potential stages where the lifecycle may be short-circuited to reduce population numbers. Based on behavioural traits of the sea louse, a light sampler was designed to collect larval lice in the field. Results in the early fall indicated that significantly more sea lice larvae were found near active farms than at reference sites. During the day, larvae were distributed in both the top and bottom waters around a shallow-water salmon farm site. Experiments in the lab indicated that individual egg strings settled at a rate of 1.0 cm/s while egg strings attached to dead gravid females settled at a velocity of 1.9 cm/s. The eggs were observed to successfully hatch under lab conditions on muddy sediments. Based on typical inshore depths and currents (10 cm/s) where fish farming occurs and the two settling velocities, we estimated egg dispersal distances of 160–300 m. Observed larval swimming speeds (1- 3 mm/s) suggested that the larvae could swim from the bottom of the water column to the surface within 3 to 8 hours. The results suggest there may be an infection cycle created within the near-field regions of the farm site itself.

Sea lice counts on Atlantic salmon farms: Comparing audit and farm staff counts

A. Elmoslemany, S. K. Whyte, K. L. Hammell, and C. W. Revie

This communication reports on the results of sea lice audits performed by the AVC Centre for Aquatic Health Sciences on commercial aquaculture sites in New Brunswick, Canada. Although the primary objective was to verify that farms were reporting similar lice counts to third-party counts, more detailed comparisons were developed to identify when lice counts were more likely to differ between the audit team and farm employees. A total of 28 sea lice audits were conducted on 16 sites from June to December 2011. During each audit, 3 to12 cages were evaluated per site, with ten fish per cage being evaluated by the audit technician and a further ten by a farm employee. Data analysis included descriptive statistics of lice counts by stage. A zero inflated negative binomial model was applied to assess the effect of audit (audit vs. farm) and season (summer vs. fall) on lice counts by stage. A three level negative binomial model was used to account for clustering on fish within cages in different sites. The results indicate that farms were generally reporting as would be expected if a third party had performed the counts but a significant difference existed between the counts recorded by the audit team and the farm employees for chalimus and preadult stages with higher abundance occurring on audit team counts most frequently. There was significant seasonal variation (autumn higher than summer counts). Finally, there was a significant clustering effect for site and cage, with most of the variation attributable to site-to-site variance.

Seafarm Pulse Guard (SPG): Protecting farmed salmon from sealice

A. Ingvarsdottir, F. Provan and H. Bredal

The Seafarm Pulse Guard (SPG) system has been developed to combat sealice at the aquaculture site. SPG has been developed in Norway and is a patented system where a skirt of electrical cables, form an electric field in the sea in close proximity of the sea pen. The system prevents mobile stages of sealice from infecting the salmon. The main objective of the project is to verify use of the SPG system and study the systems biological effects on the fish. Preliminary experiments have shown that sealice were inactivated through use of electrical pulses. These experiments have been adjusted from laboratory experiments to in situ experiments in the sea, results from the field trials will be presented. In the first phase of the field trial three different pulse systems were tested for two weeks. Atlantic salmon (control and SPG groups), were subjected to natural infection and infection levels were measured. Biological effects of the SPG system on the trial fish were analysed. The pulse system that gave the lowest infection levels of sealice was used in the long-term trial. In the long- term trial (5 months) the tolerance of the SPG system for natural influence was evaluated. In addition sealice infection levels were counted every two weeks and health condition of the fish was monitored through analysis of biochemical parameters. The results of the field trials are promising so far and in addition no negative health effects on the fish have been observed.

Topical delousing in seacages – salmon behaviour, water movements and therapeutant mixing

F. Oppedal, P. Klebert, E. Høy, R. E. Olsen, L. H. Stien and O. Folkedal

Topical delousing in sea cages is a critical point in salmon farming both for the fish welfare and production efficiency. A series of studies in both tanks and cages were performed to investigate effects of the treatment on the salmon behaviour, oxygen consumption and physiology. Major findings includes a 50% increase in oxygen consumption by the pyretroid itself, variable effects of the management procedures and physiological stress responses. Within cages, management practices affected fish behaviour such as changes in swimming speed. Following pyretroid exposure, deviating behaviours such as burst activities were frequent. Salmon movements seem important for efficient treatment exposure. Critical points in the management procedures will be discussed.

Shielding skirt for prevention of sea lice infestation on salmon in cages - experimental study on mooring loads and net deformation

A. M. Lien, Z. Volent, E. Lien and L. M. Sunde

In current Norwegian salmon farming practice, sea lice are generally controlled by using medical treatment in a closed volume, either by fitting a tarpaulin around the cage or in a well boat. Handling of the fish, in combination with the added chemicals, might be stressful to the fish and result in production loss. The operations themselves are a potential cause of escapes, time consuming, and involve a substantial cost to the fish-farmers. Initial observations indicate that a permanent tarpaulin skirt around the upper part of the cage (5 m deep), might reduce the level of infestations of lice on the salmon. Such a preventive solution will reduce

the need for delousing treatment, and thus contribute to a more sustainable parasite control. A solid skirt will however affect the cage mooring loads, the net deformation and the water flow into and around the cage. It is necessary to study these factors to prevent damage to the structures and the fish. Model scale experiments will be conducted at the North Sea Flume Tank in Hirtshals, Denmark, 26 – 28 March 2012, and will include testing on a model cage with different net solidities and skirt configurations, at different current speeds. The mooring loads, net and skirt displacement, net volume, and current inside the cage will be measured. The results will contribute to determine the technical limitations for the use of permanent skirts as a preventive disease control strategy. Results from the experiments will be presented at the Sea Lice 2012 conference.

Genetics and genomic

Genomics in Lice and Salmon (GiLS)

Ben Koop (keynote speaker)

Understanding sea lice also requires a comprehensive knowledge of their hosts. Fortunately, along with major efforts of ourselves and others on thegenomics of lice, we are part of a major collaboration that is examining the genomics of salmonids. Salmonids are a rapidly evolving species complex (66species including grayling, ciscos, whitefish, trout, char, and salmon), which are > 92% similar at the noncoding DNA level. Nearly all have significant economic, ecological, conservation, environmental and societal value. In the last 60 years, over 70,000 publications have examined fundamental and applied questions relating to fisheries, aquaculture, aquatic physiology, ecology, evolution, disease, resistance, reproduction, growth, tolerance tophysical factors, and general immunity. The Atlantic salmon genome program's overall objectives are to 1) identify all of the genes of salmonids; 2) understand the role of genetic variation and mutation for salmonid adaptation, health, and disease; 3) explore new technologies and analysis strategies to tease apart complex salmonid genetic systems and relate them to biochemical and physiological functions. The added complication of a whole genome duplication (WGD) in salmonids means that redundant systems and functional specialization must be examined in relation to interaction with sealice ectoparasites. To examine the number of single and duplicate genes (from segmental and WGD sources) we have also included most closely related non-salmonid, non-polyploid, Esox genome (Northern Pike) which helps provide an ancestral reference and a mid point for comparisons to other more distantly related fish and vertebrate genomes. Here we examine of gene duplicate retention or loss, rates of duplicate gene evolution, extensiveness of genome organizational changes in rediploidization response to WGD, the role of repeats in rediploidization, and the changes in gene and pathway expression as a result of gene duplications. WGD on specific genes, pathways, and functional ontology categories greatly impact the interactions of sea lice with salmon immune defense mechanisms.

The Salmon Louse Genome Project

Rasmus Skern-Mauritzen, Ketil Malde, Tomasz Furmanec, Richard Reinhardt, Ben F. Koop, Frank Nilsen

The salmon louse genome has been sequenced, assembled and annotated. The sequencing project and generated resources will be presented along with some preliminary results. Furthermore an introduction to the available resources will be given along with information on how to get access.

Gene regulation in the salmon louse during settlement: similarities in the ecdysteroid regulated gene cascade to other arthropods

Christiane Eichner, Torbjørn Munkejord Pedersen, Marius Hamre, Ketil Malde, Heidi Kongshaug, Sussie Dalvin, Rasmus Skern-Mauritzen, Lars Are Hamre, Frank Nilsen

The development of arthropods is controlled by ecdysteroids, the major molting hormones. The ecdysteroids are typically signaled through a heterodimeric nuclear receptor consisting of

the ecdysteroid receptor (EcR) and ultraspiracle (USP). Activation of the ecdysteroid receptor triggers gene cascades contributing to regulation of development and reproduction. In L salmonis homologous of the two partners of the ecdysteroid receptor have been found. In the L. salmonis life cycle copepodids are the link between dispersal and the parasitic life style. After settlement on a suitable host the copepodids face the host immunesystem for the first time and they have to gain sufficient energy to moult into chalimus I. In order to study copepodid biology we conducted several experiments to assess growth and development of the copepodids after they enters Atlantic salmon. To further gain knowledge about biological processes taking place in copepodids post settlement we used a 44k oligo microarray to assess gene regulation from settlement and up to moulting into chalimus I (i.e. a 6 days period). To further obtain an overview of genes involved in regulating moulting a set of genes were assessed by Q-PCR from the free-living naupli to pre-adults. The results reveal an ordered system of gene-regulation prior to moulting in a similar fashion as in other arthropods. Some of these data together with other data obtained during the parasitic copepodid period will be presented.

Development of a skin injection model for studying local and systemic responses to *Lepeophtheirus salmonis* in salmon

Laura M. Braden, Ben F. Koop, Duane E. Barker, Simon R. M. Jones

Responses elicited in salmon skin during infection with *Lepeophtheirus salmonis* result from attachment and feeding activities, including the secretion of compounds with anti-inflammatory properties. Our research explores host-specificity to *L. salmonis* by characterizing species-specific differences in skin responses. We have shown differential expression of defense-related genes in skin of resistant (pink salmon, *Oncorhynchus gorbuscha)* and susceptible hosts (chum salmon, *O. keta*; Atlantic salmon, *Salmo salar*) during infection with adult copepods1. This report describes the development and feasibility of an injection model of *L. salmonis* infection. Local and systemic expression of genes was monitored following intradermal delivery of Atlantic salmon with dopamine-elicited L. salmonis secretions (SEP). Treatment groups included <3kDa, >3kDa, <300kDa and >300kDa SEP fractions, unfractionated SEPs, and an injection control. After 12, 24 and 48 hrs, injection site and non-injection site skin was sampled for RT-qPCR and histological assessments. Quantitative expression analysis was performed on genes associated with pathways of pro-inflammatory cytokines, arachidonic acid metabolism, tissue remodeling and acute-phase proteins (APPs). Initial observations suggest activation of inflammatory mediators occurring in SEP-injected skin after 12 hrs. Utility of the intradermal injection model as a tool for understanding the salmonid response to L. salmonis infection will be discussed.

ABC transporters as factors affecting emamectin-susceptibility of salmon lice (*Lepeophtheirus salmonis*)

Jan Heumann

ABC transporters as factors affecting emamectin-susceptibility of salmon lice (*Lepeophtheirus salmonis*) Sea lice (Copepoda: Caligidae) are marine ectoparasites

representing a major threat for profitable salmon production. Parasiticides are used successfully to control lice infections, but with few drugs available, there is concern about emerging drug resistance. The in-feed delousing agent SLICE ® (Merck Animal Health) contains an avermectin, emamectin benzoate (EMB), which is effective against all parasitic stages. ABC (ATP-binding cassette) drug transporters can be potentially involved in resistance of parasites to different drugs including avermectins. Previously we isolated SL-PGY1, a salmon lice ABCB transporter showing high homology to human MDR1. However, SL-PGY1 mRNA expression was comparable in lice populations differing in EMB sensitivity. In this study, the potential involvement of other ABC transporters was investigated. Four partial cDNAs encoding for novel ABCC transporters were identified in public EST databases. Transcript levels of all transporters were similar for two strains with different EMB sensitivity and remained unaffected by EMB treatment. To elucidate further potential effects of ABC transporters on EMB toxicity, a pharmacological approach using ABC proteins inhibitors was employed. In motility bioassays, lice were challenged with EMB without inhibitor, EMB with cyclosporin A (10 μM) or verapamil (20 μM). Cyclosporin A potentiated EMB toxicity in a sensitive and a hyposensitive strain, while verapamil enhanced EMB effects only in hyposensitive lice. The results suggest ABC transporters are part of the biochemical defence against EMB, and that verapamil-sensitive transporters could be linked to differences in EMB susceptibility among lice populations.

Stressed lice and refractory responses: Host-parasite transcriptomics

Ben J G Sutherland, Kim W Koczka, Simon R M Jones, Ben F Koop:

Lepeophtheirus salmonis infections can have different outcomes depending on host species and development stage. Louse population propagation may be strongly influenced by environmental factors, such as salinity and temperature. Using transcriptomics, we investigate these factors to better understand the potential outcomes of infections for individuals and ecosystems. Chum (*Oncorhynchus keta*), pink (*O. gorbuscha*) and Atlantic salmon (*Salmo salar*) were experimentally challenged with Pacific *L. salmonis* copepodids. Initial infection intensities were highest in chum and lowest in pink salmon. Skin and anterior kidney were sampled at 3, 6 and 9 days post infection for transcriptome profiling. Results indicate strong differences in responses among all species at local and systemic levels; responding gene functions include iron homeostasis, immunological response and energy consumption. Serum prostaglandin E2 levels also indicate differences among responses. Transcriptome profiling was performed on copepodids exposed to varied environmental conditions. Temperature changes (range: 4-16 °C) did not strongly affect gene expression after 24 hours, whereas hypo-salinity drastically affects gene expression within 5 parts per thousand (ppt) of normal seawater. Fine scale resolution of gene expression changes was acquired through exposures with salinity decreasing by single ppt increments from 30 to 25 ppt. Differentially expressed genes were clustered by similar expression patterns and characterized through enrichment analysis. Changes in cellular chaperone expression indicate early compensating mechanisms; cluster patterns and chromatin modifier expression changes indicate highly coordinated transcription programs. Comparing these responses to those of pre-adult Pacific lice treated

with emamectin benzoate may allow for relative stress quantification of salinity and temperature.

Transcriptomic analysis of the salmon louse

Rolf B. Edvardsen, Stig Mæhle, Tomasz Furmanek, Ketil Malde, Bjørn Olav Kvamme, Sussie Dalvin, Rasmus Skern-Mauritzen:

Salmon louse (*Lepeophteirus salmonis*), is a serious threat to wild populations of salmon and an important pathogen in salmon aquaculture and more knowledge about the sea lice biology, including the transcriptome, is important in the work towards control of this parasite. We have designed a 44k oligo microarray based on ESTs. This covers about 7000 salmon *L. salmonis* genes. The microarray is a powerful molecular tool to obtain an overview of the transcriptome. We have investigated subcuticular, brain, testis and ovary tissues in adult louse for a general transcriptome overview. In addition, we have taken a closer look at the transcripts in the gut. We have compared which genes that are up- and down-regulated between the louse that have been starved or not, and between female and male louse. In depth knowledge about the salmon louse gut biology is of great importance for the development of vaccines. The results reveal new and interesting findings that will be presented. The data obtained will be an important asset for further work on L. salmonis in general and for vaccine development in particular.

Posters

Transmembrane proteins in the intestine of salmon louse

S. Dalvin, B.O. Kvamme, Mæhle, S. and R. Skern-Mauritzen

Background: Actively reproducing adult females require large energy uptake and is highly dependent on digestion of blood, slime and skin from the host (salmonoids) to produce as many offspring as possible. Chemicals or vaccines, which are able to disturb the function of the intestine and reduce the uptake of food, will efficiently reduce the reproductive output. Methods: Transmembrane proteins found in the salmon louse intestine, was identified by in silico analysis, microarrays, in situ hybridization and transcriptome information. Molecular characterization and evaluation was performed on genes which appeared as good targets for vaccine development was evaluated using bioinformatic tools and RNA-interference. Results: 24 genes were identified based on sequence analysis. Six of these genes were found to be expressed in the intestine of adult females. RNA interference studies revealed that digestive processes and reproduction was dependent on expression of some of these genes.

A Pacific sea lice reference genome

J. S. Leong and B. F. Koop

Fundamental sea lice genomic resources need to be expanded. *Lepeophtheirus salmonis* are comprised of distinct species in the Pacific and Atlantic ocean. To further efforts in understanding the relationships between these distinct species, we report the assembly of reference transcriptomes. Assembly strategies will be discussed. For Canadian Pacific *L. salmonis*, 64,666 ESTs + ~294M Illumina Hi-Seq PET reads were assembled into a

Transcriptome Shotgun Assembly (TSA) of 35,589 contigs. 16,706 contigs were assembled from 9,578 ESTs and ~303M Illumina PET reads, for Canadian Atlantic *L. salmonis*. These high-quality reference transcriptomes form the basis for comparisons between other groups of known sea louse as well as identifying gene expression patterns associated with environmental, physiological and developmental changes. Comparisons to *Caligus clemensi* (14,821 ESTs), *C. rogercresseyi* (32,135 ESTs) and *Lernaeocera branchialis* (16,441 ESTs) show that Caligus are a very old group with deep divergent lines. To expand on genomic resources, a 88x next-generation whole genome shotgun (WGS) assembly of Pacific *L. salmonis* was optimized using ~501M reads (80x) from Illumina together with 13.8M reads (8x) from 454. The resulting assembly produced 149,469 contigs having an N50 9.74 kb. This new high-coverage assembly provides a framework for further insight into discovering genes and gene organization as well as a comparison with a nearly completed Atlantic salmon louse genome. Genomic studies of individual sea lice reveal a very high diversity of bacterial, fungal, and protozoan co-habitants that may likely affect its physiology, reproduction, development, and response to the environment.

Composition and high abundance of transposable elements in Atlantic and Pacific *Lepeophtheirus salmonis* genomes

D. Minkley, B. Koop and J. Leong

Transposable elements (TEs) are fundamentally important to the evolution of both genome structure and regulation. TE insertions can induce modifications in genes and gene regulatory networks, playing a role in adaptive evolution and facilitating directed mutation. The presence of TEs frequently enables large-scale genome restructuring and is involved in speciation. Using in silico analyses we investigate the composition and abundance of TEs in the genomes of Atlantic (Norway) and Pacific (Canada) subpopulations of *Lepeophtheirus salmonis*. Repeats were identified from draft genome assemblies of the two *L. salmonis* subpopulations by extending the REPET and RepeatModeler pipelines. TE families were refined through in-house scripts, BLAST searches and manual classification based on existing TE libraries. Two preliminary repeat libraries were created: the Atlantic library containing 4,824 families and the Pacific library containing 3,700 families. The RepeatMasker program was used to investigate genome repeat content. Class II DNA transposons were the most abundant in both genomes. Class I retrotransposons were also identified. It was determined that 54% of the Pacific genome and 58% of the Atlantic genome were composed of TEs. Unlike in Atlantic salmon (*Salmo salar*), the majority of TE insertions are not instances of a few abundant families, but instead constituents of hundreds of families. The repeat composition of the two subpopulations was compared and found to be highly similar. We will comment on the unexpectedly high abundance of TEs in the *L. salmonis* genome, and discuss their ability to effect rapid evolution and parasiticide resistance in this economically important aquaculture parasite.

A study of gene expression in Atlantic *Lepeophtheirus salmonis* populations with differing susceptibilities to emamectin benzoate

S. Carmichael, A. Tildesley, J. Ireland, J. B. Taggart, P. Skuce, J. E. Bron and A. Sturm

Lepeophtheirus salmonis (Krøyer, 1837) is an ectoparasitic copepod that has emerged as a serious problem for cultured and wild salmonid populations. The annual cost of sea lice to global salmon farming was estimated at $480 million in 2006 and is increasing. Emamectin benzoate has been one of the most successful antiparasitic agents to be used against L. salmonis infections of Atlantic salmon, being employed as a 0.2% in-feed pre-mix (SLICE®). In recent years, increased incidence of reduced *L. salmonis* sensitivity to this treatment has been experienced. The transcriptomic responses of two *L. salmonis* populations, displaying differing susceptibilities to emamectin benzoate, were studied using a custom sea louse 15K oligonucleotide microarray and RT-qPCR. Sea lice were sampled from both populations after 1 and 3 hours in vitro exposure to 0.2 µg mL-1 emamectin benzoate. Bioinformatic analysis identified a large group of genes that were significantly down regulated in the hyposensitive lice strain and decreased gene expression was also evident in genes from the susceptible strain after 1 hour exposure when compared to control lice. This custom microarray provides a tool for the identification of key biomarkers which could to be utilised in a routine RT-qPCR assay for establishing the degree of susceptibility to emamectin benzoate in louse populations.

Biological and transcriptional consequences of infection with *Facilispora margolisi* (Microsporidia: Crustaceacida) following vertical transmission among larval salmon lice *Lepeophtheirus salmonis*

S. R. M. Jones, B. J. G. Sutherland and B. F. Koop

Facilispora margolisi occurs in *Lepeophtheirus salmonis* in the northeastern Pacific Ocean. The consequences of infection with this newly described microsporidian are not known. Vertical transmission was confirmed in four larval culture trials in which *F. margolisi* was only detected in copepodid progeny from infected females. Infected and uninfected copepodids were separately pooled and used to determine effects of *F. margolisi* on copepod infectivity and gene expression. Microsporidian infection had no effect on infection and development of *L. salmonis* on laboratory-exposed chum salmon (*Oncorhynchus keta*). Pools of infected and uninfected copepodids were further sub-divided into 14 groups of approximately 75 copepodids maintained in aerated seawater. Seven infected and seven uninfected groups were incubated with 1 µg L-1 emamectin benzoate (EB) while the remainder were sham exposed. After 24 h, copepods in each group were pooled and stored at -80°C. Gene expression among groups was compared by using a 38K oligo-array and by quantitative reverse transcriptase PCR. To investigate the relative level of transcriptome responses to microsporidia and/or EB, differentially expressed genes and enriched functional categories from each experimental condition were analyzed. Principle component analysis suggested infection accounted for 33% of variation in microarray data and cluster analysis identified a synergistic effect of EB and microsporidia on the expression of genes belonging to the Gene Ontology category Protein Folding. We discuss possible implications of F. margolisi infections on the physiology of L. salmonis, including susceptibility to EB.

Emerging issues

Novel experimental approaches to facilitate development of new control measure towards sea lice

Frank Nilsen (Key note speaker)

Current salmon louse control is strongly dependent on a few anti salmon louse medicine. Emerging development of drug resistance has made salmon louse control more difficult and there is a strong need for new medicines to break the resistance and novel non-medical treatment tools. Novel treatment methods like vaccines are difficult to develop for complex parasites like sea lice. A main challenge is to identify protective antigens among the many thousands of candidate genes. Furthermore, parasites like the salmon louse can only grow on their host (i.e. salmonids) and this puts strong limitation on experimental design and maintenance of lice in the laboratory. Sequencing of the salmon louse genome is completed and the assembled genome will be annotated and available to the public soon. This will be a large boost to facilitate research on salmon louse both at the basic level but also a key resource for developing new control tools in the future.

To facilitate both research on salmon louse biology and to increase the likelihood for developing new salmon louse control methods, a set of molecular and experimental tools have been established. In this presentation, some of the new research tools will be presented and some examples on new biological insight will be given.

Salmon Lice Population Dynamics Modeling with Applications to Treatment Strategies

Marit Stormoen, Brad Schofield

In order to improve treatment strategies for Salmon lice (*Lepeophtheirus salmonis*) infestations, it is necessary to understand the dynamics of the host-parasite interactions. Dynamic population models for lice on farmed fish have been developed in recent years. In this work, a dynamic model incorporating density dependent sexual reproduction is presented. The model uses a statistical approach to determine the likelihood of reproduction based on the abundance of adult lice. This effect, in combination with the dynamics of population growth, leads to a system that exhibits bifurcation, that is, the system is stable for certain values of lice abundance but is unstable (population grows exponentially) for other abundance values. This result has implications for the planning of treatment strategies. If lice abundance can be held below this bifurcation level, large-scale reproduction (with resulting reinfection after treatment) may be prevented. Continuous daily treatment options, such as wrasse, may be implemented to achieve this. The model presented will be used in simulations for analyzing the effects of different medicinal treatment strategies by analyzing and including the effects of various common treatments in the dynamic model. For instance a thorough knowledge of the growth of mobile stages may allow us to predict the upcoming surge of gravid females and implement treatments before a massive propagation has been allowed to take place, this may help reduce the necessary treatments in the long run.

Introducing Sealice Explorer – a diagnostic e-taxonomy system for sealice

P M Hayes and G A Boxshall

Sealice first emerged as a disease problem in aquaculture industry in the 1970s. However, the number of species impacting farmed finfish has expanded rapidly and now includes species that can spread into brackish water and infest coastal farms in SE Asia where tilapia culture is important. Wherever a new finfish is taken into culture, sealice problems emerge. Culturing exotic fish species does not circumvent the lice problem: local sealice can switch from natural hosts onto the farmed fishes within a few years (e.g. *Caligus rogercresseyi* in Chilean salmonid farms). In addition there are examples where adult sealice, but never larvae, are found on cultured fish. Where are their larvae? The identification of sealice can be problematic and laborious, particularly for non-specialists. To improve our ability to identify sealice we have developed an online Scratchpad system – Sealice Explorer (http://sealice.myspecies.info). This provides an interactive diagnostic system to aid routine identification of adult sealice, and offers training in identification and description techniques. The current version includes an interactive key to species of *Caligus* (260+ valid species), based on morphological characters of both sexes, supported by schematics and descriptions. This system will ultimately cover all species within the 34 valid caligid genera. Sealice Explorer also provides a platform for users to access and disseminate data on sealice – including information on sealice biology and larval development, host and distribution data, a photographic archive and links to publications and DNA sequence data. We aim to build a powerful tool for fish health management and research alike.

Search of vaccine candidates against salmon louse

Christer R. Wiik-Nielsen, Celia Ridaura and Søren Grove

Alternative solutions, to today's chemical treatment in reducing the number of salmon louse (*Lepeophtheirus salmons*), are urgently needed. In this project we aim at identifying antigen candidates to be used in vaccines against the salmon louse. Our strategy is to look for 'weak points' in the digestive system of the parasite. The parasite is a blood/mucus feeder, and need mechanisms to protect themselves against harmful substances in the ingested blood, such as complement factors. To identify parasite antigens we use a method called phage display. The technique involves preparation of a parasite protein library expressed on bacteriophages. The proteins in the library are then tested for possible binding to factors isolated from salmon blood (e.g. complement). Louse proteins binding to blood factors may be specific inhibitors of these and hence part of the louse protective system. Identified proteins will be good antigen candidates which are then further characterised and tested in RNAi experiments. So far one possible candidate, a parasite protein that binds to complement factor C3, has been identified. In addition, preliminary data from haemolytic analyses has shown that louse proteins are able to inhibit the complement reaction.

Variability between full-sibling famillies of salmon lice (*Lepeophtheirus salmonis*) in common garden experiments

Lina E.R. Ljungfeldt, Kevin A. Glover, Per Gunnar Espedal and Frank Nilsen:

In order to improve treatment strategies for Salmon lice (*Lepeophtheirus salmonis*) infestations, it is necessary to understand the dynamics of the host-parasite interactions. Dynamic population models for lice on farmed fish have been developed in recent years. In this work, a dynamic model incorporating density dependent sexual reproduction is presented. The model uses a statistical approach to determine the likelihood of reproduction based on the abundance of adult lice. This effect, in combination with the dynamics of population growth, leads to a system that exhibits bifurcation, that is, the system is stable for certain values of lice abundance but is unstable (population grows exponentially) for other abundance values. This result has implications for the planning of treatment strategies. If lice abundance can be held below this bifurcation level, large-scale reproduction (with resulting reinfection after treatment) may be prevented. Continuous daily treatment options, such as wrasse, may be implemented to achieve this. The model presented will be used in simulations for analyzing the effects of different medicinal treatment strategies by analyzing and including the effects of various common treatments in the dynamic model. For instance a thorough knowledge of the growth of mobile stages may allow us to predict the upcoming surge of gravid females and implement treatments before a massive propagation has been allowed to take place, this may help reduce the necessary treatments in the long run.

Posters

A new copepod-specific trypsin mutant as an antigen candidate for a vaccine against sea lice

M. Patricio, S. Rodrigo, M. Carlos, B. Catalina and T. Jaime

Vaccine development against sea louse represents a preventive strategy to reduce parasitism in salmon farming, complementary to current control methods. Rational vaccine development depends on the identification of both exposed lice antigens and of so called “concealed antigens” (CA), which corresponds to parasite’s molecules which does not normally interact with fish immune system, but their neutralization affects parasite’s physiology, Sea lice’s gut proteases participate actively in parasite’s feeding process and also in blocking host’s immune response, making them attractive candidates for CA. In our laboratory we have identified six different trypsins, which exhibit different cycle-specific transcription profiles. One of them (trypsin-cop, specific of Copepodid stage), presents a sequence about 70% amino-acid identity with a common trypsin found in databases (*Caligus rogercresseyi* trypsin-1, ACO11732), but presenting two specific mutations in its catalytic triad. These mutations have an important effect in the protease catalytic mechanism and are consistent with reports of inactive, wild type, serine-proteases found in other parasites. In silico analysis and homology modeling between Trypsin-cop and trypsin-1 show amino-acid conservation in their catalytic pocket, but substantial differences in surface residues. In order to confirm absence of protease activity, we have cloned the cDNA sequence of trypsin-cop and trypsin-1 in a yeast

expression system, recombinant proteins were purified and activity was analyzed by AzoCasein method. In order to evaluate their antigen potential, injectable and oral vaccines were formulated with recombinant trypsins and applied in rainbow trout to evaluate anti-trypsin IgM and IgT titers in blood and skin mucus.

Influence of different materials on the concentration of delousing agents in seawater

K. Olli Helgesen, R. Landsem and T. E. Horsberg

A 24 hours bioassay for field tests of sensitivity of salmon lice against the pyrethroids deltamethrin and cypermethrin, the organophosphate azamethiphos and the avermectin emamectin benzoate, has been developed. To select the most suitable material for the assay, the concentration of the agents in seawater in containers of different materials was tested over a 24 hours period. One-liter containers made of low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polystyrene (PS), Teflon and glass were tested. Each container was filled with exactly 1 liter of filtered sea water and spiked with appropriate concentrations of deltamethrin (AlphaMax), cypermethrin (Betamax), azamethiphos or emamectin benzoate. Samples were taken immediately after spiking and 24 hours later and analyzed by LC-MS/MS or HPLC-fluorescence. All tests were performed in triplicates. The tests demonstrated that the pyrethroids disappeared almost completely in LDPE, HDPE and PP during 24 hours. There was a notably lower reduction in PS, and least in Teflon and glass. For the other active substances, the material of the container was less significant. On this basis, glass flasks were chosen as the material for the assays.

Characterizing the interaction of emamectin benzoate with P-glycoprotein in sea lice, *Lepeophtheirus salmonis*

O.O. Igboeli, M.D. Fast, J.F. Burka

Over-reliance on emamectin benzoate (EMB), a macrocyclic lactone (ML), for the control of sea lice in salmon farms has led to decreased sensitivity to the parasiticide and a consequent decline in its use. P-glycoprotein (P-gp), a member of the ATP-binding cassette (ABC) transporters, has been strongly linked to resistance development to the ML family of parasiticides in arthropods and nematodes. We investigated the interaction of EMB with P-gp using a competitive inhibition test as well as an assay for ATPase activity. Ivermectin, a known P-gp substrate, was used as a positive control in the assay for ATPase activity. EMB and ivermectin both decreased baseline vanadate sensitive ATPase activity with 50% inhibition of 26.35 and 0.14 μM respectively. Both compounds inhibited maximum vanadate-sensitive ATPase activity with IC50 of 7.82 μM for EMB and 4.98 μM for ivermectin. In a bioassay, concomitant exposure of adult male and female *Lepeophtheirus salmonis* to the ABC transporter inhibitor, verapamil, along with EMB caused > 2-fold higher % mortality than when the parasites were exposed to the parasiticide alone. When compared to adult male *L. salmonis*, the adult females were more sensitive to EMB, with or without added verapamil. Our results strongly suggest that P-gp is involved in EMB detoxification in *L. salmonis*.

Learning from the past and building for the future: The control of sea lice

J. G. McHenery

Since nearly the inception of salmon farming in Europe, sea lice have been a problem and arguably remain the biggest disease issue in global salmonid aquaculture. The introduction of medicines has been critical in enabling the industry to control these parasites, and yet no new class of therapeutants has been introduced since the 1990s. Novartis Animal Health, as its predecessor company Ciba-Geigy, was the first company to gain a Marketing Authorisation for a medicine for the control of lice in salmon and remains the only company to have ever had more than one medicine authorised and available to the vet and farmer. In this paper, we will consider how the Aqua parasiticide market relates to other animal health sectors and the development philosophy of Novartis Animal Health. We will examine the processes involved in developing a product from initial screening, through trialling to Marketing Authorisation and ongoing support, with examples from our past and ongoing programmes.

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